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Author(s)	Thavaroj, Wichulada; Pansawat, Nantipa; Konno, Kunihiko
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Thermal denaturation profiles of catfish and tilapia myofibrils as affected by pH for heating.

Running title: Thermal denaturation of catfish myosin

Wichulada Thavaroj[*1], Nantipa Pansawat[*2], Kunihiko Konno[*1,*3]

[*1] Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

[*2] Faculty of Fisheries, Kasetsart University, Bangkok, 10900 Thailand

[*3] Corresponding author

E-mail: konno@fish.hokudai.ac.jp

Abstract: Thermal denaturation profiles of catfish myosin when heated as myofibrils (Mf) were compared with those of tilapia ones. Ca^{2+}-ATPase inactivation rate of catfish myofibrils was the same as that of tilapia myofibrils. The conclusion was the same with isolated myosin. Catfish Mf was clearly distinguished form tilapia Mf in subfragment-1 (S-1) and rod denaturation. A quick denaturation of rod relative to S-1 was characteristic for catfish Mf while a slower denaturation of rod relative to S-1 was the pattern of tilapia Mf. These patterns were greatly affected by the pH for heating. With increasing the pH for heating, rod denaturation was accelerated and oppositely suppressed by lowering the pH for both Mf. Tilapia Mf showed a similar S-1 and rod denaturation pattern to catfish Mf by increasing 1 pH unit. For example, a pattern with catfish Mf at pH 7.5 was similar to one with tilapia Mf at pH 8.5. Less rigid filament structure of catfish Mf than tilapia Mf was demonstrated by studying chymotryptic digestion at various pH. Accordingly, different S-1/rod denaturation pattern for two fish species was explained by the different rigidity of myosin filaments.

Keywords: catfish, myosin, denaturation, chymotryptic digestion, tilapia, myosin filaments

Introduction

Various species of catfish are cultured in Thailand. Among them, a hybrid, *Clarisas macrocephalus x Clarias gariepinus*, is the most abundantly cultured species and economically valuable due to its high growth rate, wide environmental tolerances, resistant to infection by bacteria, better taste, and high yield [1]. The cultured fish is exported to Western countries especially to United State of America in a form of frozen fillet and frozen mince. The fish is also consumed domestically. Total production of catfish in 2010 was more than 1,500 tons with a value of 3 million US$. Catfish meat is characterized by white and less greasy with delicious taste. In spite of the importance of the fish species in Thailand, there is little information on the properties of myosin, especially on its biochemical functions and stability. As myosin is a major protein in muscle, its denaturation affects a quality of the final product. For example, myosin denaturation in Surimi reflects the thermal gel properties. Thus it seems reasonable to think that myosin denaturation in fish meat determines its quality. The information on the stability and denaturation of myosin of the fish would be valuable for the evaluation of the quality of fish fillet during its storage.

Tilapia is another important fish species cultured in Thailand. It is believed that myosin from tilapia is one of the most stable myosin ever isolated from fish muscle, which is explained by high water temperature for its habitation [2, 3]. Catfish and tilapia are cultured under similar environmental conditions, especially water temperature. Catfish myosin is presumed to have a similar stability to tilapia myosin. Another factor that affects myosin stability in myofibrils is the magnitude of protective effect of F-acin. It is reported that the stabilization extent by F-actin binding is fish species-dependent [4]. To understand the stability in meat, thermal stability of myosin

in myofibrils as well as in isolated form should be studied by measuring thermal inactivation of Ca^{2+}-ATPase. Chymotryptic digestion is a very useful technique to study myosin denaturation occurring on subfragment-1 (S-1) and rod portion of myosin. It has also been reported that S-1 and rod denaturation proceed differently and the pattern is fish species-specific [4]. The rod denaturation studied by chymotryptic digestion is based on the fact that native myosin in filamentous form in myofibrils is cleaved selectively only at S-1/rod junction. Cleavage at other sites within rod portion is the index of rod denaturation, which is easily detected by the decreased rod production in SDS-PAGE. In other words, the index detects structural changes of myosin filaments upon heating of myofibrils. Accordingly the index should be re-evaluated by considering the myosin filament structure. Up to now, myofibrils were usually suspended in 0.1 M NaCl (KCl), 20 mM Tris-HCl (pH 7.5) according to the original preparative method by Katoh *et al.* [5], and heating was conducted basically in the same medium. All of the conclusions led were ones obtained under the conditions.

In the present study, thermal stability of catfish myosin in isolated form and in myofibrils was studied by comparing Ca^{2+}-ATPase inactivation rates with that of tilapia. Myosin denaturation in myofibrils of catfish was studied by S-1 and rod denaturation pattern. S-1 and rod denaturation in myofibrils was re-examined by changing pH for heating to understand the involvement of myosin filament structure in the pattern. Different rigidity of myosin filaments in catfish and tilapia myofibrils was further studied by monitoring the change in the chymotryptic digestion pattern at various pH.

Materials and Methods

Myofibrils were prepared from dorsal muscle of hybrid catfish *Clarisas macrocephalus x*

Clarias gariepinus and from tilapia *Oreochromis niloticus x mosambichus* as described earlier [5]. Myofibrils were suspended in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5). Myosin was prepared from the myofibrils by applying the ammonium sulfate fractionation in the presence of 2 mM $MgCl_2$-ATP as reported [6]. Myosin was finally dissolved in 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5). Thermal denaturation of myosin was monitored by following the Ca^{2+}-ATPase inactivation. Heating temperature was optionally changed dependent on purposes. Ca^{2+}-ATPase was assayed in a medium of 0.5 M KCl, 25 mM Tris-maletae (pH 7.0), 5 mM $CaCl_2$, and 1 mM ATP at 25°C. The inactivation rate (sec^{-1}) was calculated by assuming the inactivation as the first order reaction kinetics.

Myosin denaturation in myofibrils was also studied by chymotryptic digestion. Decrease in the amount of S-1 and rod produced and monomeric ones were the indices for the denaturation of respective regions. Heated myofibrils were digested at 20°C for 60 min in a medium of 0.05 M NaCl, 20 mM Tris-maleate (pH 7.0) and 1 mM EDTA by using 1/200 (w/w for catfish) and 1/400 (w/w for tilapia) of chymotrypsin so as to cleave myosin into S-1 and rod [7,8]. When myofibrils were heated at varied pH, the pH of the solution was adjusted to 7 by adding either 1 M Tris or maleic acid with the dilution of NaCl to 0.05 M. The volume of Tris and maleic acid solution to be added was determined in advance. Fragments in the digestive recovered in the supernatant at 40% saturated ammonium sulfate in the presence of 2 mM Mg-ATP were referred as monomeric compounds. These were estimated by measuring the staining intensity of the corresponding bands on SDS-PAGE. SDS-PAGE was performed according to Laemmli [9].

Results

Thermal stability of catfish myosin and myofibrils with comparison to that of tilapia

SDS-PAGE patterns of myofibrils and myosin of catfish and tilapia are shown in Fig. 1. Patterns of myofibrils for the two species were similar to each other (Fig. 1a). The band below actin was identified as tropomyosin by its behavior in ammonium sulfate fractionation myofibrils, namely it was recovered in the supernatant at 40 % saturation with and without Mg-ATP addition (data not shown). The migration distance of tropomyosin for two samples was also the same. Purified myosin gave the information on the position of light chain (LC) components (Fig. 1b). Both myosin samples showed three LC bands termed as LC1, LC2, and LC3. The mobility of LC2 and LC3 for catfish and tilapia was the same, but the LC1 in catfish myosin showed a little longer migration distance than the corresponding one in tilapia myosin (Fig. 1b). It is well established that one of LC components has an ability to bind Ca^{2+}, and the removal of Ca^{2+} from the LC is needed for the cleavage of myosin into S-1 and rod [7]. Among the LC components, LC2 in catfish and LC3 in tilapia disappeared upon digesting myofibrils in the presence of EDTA (see Fig. 4). Although the LC composition for catfish and tilapia looked similar, the size of Ca^{2+} binding LC was different for the two myofibrils.

Thermal stability of catfish myosin in myofibrils was studied by following Ca^{2+}-ATPase inactivation. A faint actin band was seen with myosin preparation (Fig. 1b), but the its effect was negligible because slow inactivation phase derived from myosin in actin bound form was not detected. Ca^{2+}-ATPase inactivation profile of catfish myofibrils in 0.1 M NaCl, 20 mM Tris-HCl (pH7.5) upon heating at 40˚C was compared with that of tilapia (Fig. 2a). There was no difference in the inactivation rates between two samples, i.e., stabilities of myosin with full protection by F-actin were the same.

Thermal stability of myosin itself for two species of fish was also the same when heated at 30˚C in 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5) (Fig. 2b). Thus, it was concluded that catfish myosin is as stable as tilapia myosin and that the protective effect by F-actin observed with catfish and tilapia myofibrils were also the same.

The conclusion was confirmed by measuring their thermal inactivation rates of Ca^{2+}-ATPase at varied temperatures. The rates at 0.1 M and at 2 M NaCl were measured to access the rates with and without protection by F-actin, respectively (Fig. 3). A complete loss of protection by F-actin in myofibrils at 2 M was proved because the rate obtained was the same as that of myosin itself (data not shown). Both myofibrils gave identical lines under respective NaCl concentrations in Arrhenius plot. The rates at 0.1 M and 2 M calculated from the lines in the figure gave the stabilization extent upon F-actin binding. The extent of approximately 100 times for the two species of fish was calculated at the same temperature.

S-1 and rod denaturation profiles of catfish and tilapia myosin when heated as myofibrils.

To investigate rod denaturation as well as S-1 when heated as myofibrils, change in the chymotryptic digestion pattern upon their heating was studied. We also studied whether the fragments produced from the heated myofibrils are all monomer because rod found in the digest sometimes contains aggregated one [4]. Aggregates are well removed from the digest by applying ammonium sulfate fractionation at 40 % saturation, in which monomeric rod was recovered in the supernatant. SDS-PAGE patterns of the digests of heated myofibrils and their supernatant at 40% saturation were both shown in Fig. 4. Myosin in unheated myofibrils was selectively cleaved into

S-1 and rod for both species. The amount of S-1 and rod produced from myofibrils decreased with duration for both fish species. Apparently, decreasing profile of S-1 was almost the same with two myofibrils. However, there was a significant difference in the rod production. A very small amount of rod was produced from the heated catfish myofibrils and was all monomer. In contrast, a quite large quantity of rod was produced from the heated tilapia myofibrils, but the rod contained aggregated one. A clear band locating above rod and several bands migrating below S-1 were the new fragments detected with the digest of heated catfish myofibrils. Practically all of the newly detected fragments as well as S-1 and rod were all monomer (Fig. 4a-s). Although the data were not shown, the band above rod was not recovered in the supernatant in the absence of Mg-ATP indicating that the fragment is present is F-actin bound form under the conditions, whereas the bands below S-1 were recovered in the supernatant indicating that the fragment does not contain myosin head region. It was suggested that a preceded decrease of rod production than S-1 with catfish myofibrils was the consequence of the additional cleavage of myosin into heavy meromyosin (HMM)-like and light meromyosin (LMM)-like fragments as proved with carp myofibril digest [11]. LMM-like bands once produced decreased with duration probably due to degradation into much shorter fragments. Digestion of heated tilapia myofibrils also generated several new bands located above rod, while there was no production of bands migrating between S-1 and actin band. None of the bands detected above rod were recovered in the supernatant at 40 % saturation, i.e., they were aggregated fragments. Moreover, amount of monomeric rod was clearly smaller than that found in the digest indicating that rod contained aggregated ones. Due to different behavior of rod production upon heating between two myofibrils, apparently different S-1 and rod denaturation patterns were generated. When the rod production was the index, slower and faster decrease of

rod relative to S-1 decrease characterized tilapia and catfish myofibrils, respectively. When the monomeric rod was the index, similar decrease of monomeric rod to S-1 was the profile of tilapia myofibrils. The pattern for catfish was the same as studied with amount of rod produced.

The amounts of S-1 and rod produced from the heated myofibrils and their monomeric form were all estimated (Fig. 5). All of S-1 produced was monomeric irrespective the amount. Ca^{2+}-ATPase inactivation and S-1 content decreased in parallel for both fish myofibrils. It was confirmation that S-1 was generated only from myosin that retains Ca^{2+}-ATPase active [10]. As expected in Fig. 4, rod produced from catfish myofibrils was all monomeric, and the decrease was much quickly than S-1. Incubation for 80 min reduced the S-1 production to approximately 45 %, whereas rod production was as low as 20 % (Fig. 5a). On the other hand, rod production decreased slowly with heated tilapia myofibrils, and was much slower than S-1 production. Meanwhile decrease of monomeric rod was almost the same or a little slower than S-1. Incubation of tilapia myofibrils for 80 min decreased the rod production only to 60%, whereas monomeric rod was as low as 38% (Fig. 5b).

S-1 and rod denaturation patterns as affected by the pH for heating.

Denaturation of rod portion of myosin in myofibrils was studied by using chymotryptic digestion, i.e., exposure of additional cleavage sites within rod portion in myosin filaments was the index of rod denaturation. It is reported that filament forming ability of myosin differs from fish species to species [11]. Moreover, rigidity of myosin filaments is affected by several factors. Mg^{2+} addition and lowering pH promote filament formation by reducing negative charge of myosin rod surface [12]. The promotive effect

of reducing negative charge on filament formation was proved by chemical modification experiment, i.e., incorporation of methyl glycine into carboxyl groups as catalyzed by N-(3-dimethaminopropyl)-N'-ethylcarodiimide promoted filament formation by myosin rod [13].

We intended to study the effect of rigidity of filament on S-1 and rod denaturation pattern. For the purpose, pH for heating myofibrils was changed. As shifting pH from neutral pH toward both directions enhances myosin Ca^{2+}-ATPase inactivation [14], the pH range for heating was chosen so as not to change the inactivation rate so significantly, i.e., from pH 6 to pH 9. Myofibrils of catfish and tilapia suspended in 20 mM buffer at various pH containing 0.1 M NaCl were heated at 40°C. The myofibrils were digested under the identical conditions of 0.05 M NaCl, pH 7.0 and 1 mM EDTA. Amounts of rod produced, monomeric rod, and monomeric S-1 were estimated (Fig. 6). S-1 production decreased to 50% roughly in 60 min at pH 7.5, while the decrease was slightly faster at pH 6.0 (40 min) and at pH 9.0 (25min) for catfish. Decrease of S-1 production by 50% occurred in 60 min at pH 7.5, in 25 min at pH 6.0 and pH 9.0 for tilapia. As S-1 production is closely related to ATPase inactivation, the results were basically consistent with the results by Hashimoto *et al.* [14]. However, decrease of rod production was significantly affected by the pH for heating. Rod production decreased very slowly at acidic pH, and oppositely it decreased very quickly at alkaline pH. The trend was commonly observed with both species of fish myofibrils. Decrease of 50% rod production occurred in more than 90 min at pH 6, and in less than 5 min at pH 9.0 for catfish. Similarly, the event occurred in more than 90 min at pH 6, and in 10 min at pH 9.0 for tilapia. Production of aggregated rod in acidic pH region was also a common event observed although the event was more prominent for tilapia, i.e., decrease of rod production was slower than that of monomeric rod. The presence of

aggregated rod at acidic pH indicated that myosin filaments became very rigid and resistant to chymotryptic attack even in aggregates. Although S-1 and rod denaturation patterns at pH 7.5 for catfish and tilapia were different significantly, respective patterns at pH 6.0 and at pH 9.0 for both myofibrils were similar. At pH6.0, slower rod denaturation than S-1 and the production of aggregated rod were commonly observed. Similarly, the profiles at pH 9.0 for catfish and tilapia myofibrils were characterized by a quick rod denaturation than S-1 with no aggregated rod production. Comparing the pattern at various pH for two myofibrils, we noticed that a similar S-1 and rod denaturation pattern was obtained at different pH for catfish and tilapia. For example, the pattern of catfish myofibrils at pH 7.5 was similar to one observed at pH 8.5 and 9.0 with tilapia myofibrils. Catfish myofibrils required to lower the pH for heating by roughly 1 pH to give a similar pattern to tilapia myofibrils. Oppositely, the pattern of tilapia myofibrils at pH 7.5 was generated when catfish myofibrils were heated at pH 6.5. Fortunately, heating myofibrils at pH 7.5 was the best conditions for detecting different S-1 and rod denaturation profiles between catfish and tilapia.

Different myosin filament structure in catfish and tilapia myofibrils was studied by comparing the susceptibility of filaments in these two myofibrils to chymotryptic attack at various pH. Myofibrils of catfish and tilapia were digested at pH between 6 and 9. Selective cleavage of myosin into S-1 and rod was found below pH 7.0 for both myofibrils as expected. However, digestion at alkaline pH generated fragments migrating between actin and S-1 together with S-1 and rod. As the production of the fragments was accompanied by the decreased rod production, additional cleavage within myosin rod probably at HMM-LMM junction was suggested. To characterize the digestion patterns of catfish and tilapia, the patterns at pH 8.0 were presented (Fig. 7). Myosin in tilapia myofibrils was quite selectively cleaved into S-1 and rod (Fig. 7b).

Negligible amount of other fragments were produced. The amount of rod and S-1 produced was reasonable when molecular weights of these two fragments were considered. On the other hand, catfish myofibrils gave different pattern from tilapia myofibrils (Fig. 7a). Although major fragments produced in an early phase of digestion were S-1 and rod, an additional faint band above rod and several bands between S-1 and actin were formed in latter phase. These fragments were the same as found in the digest of heated catfish myofibrils (Fig. 4). Amount of rod was much smaller than that of S-1 in the digest for 60 min. It was thus demonstrated that amount of rod was determined by the filament structures. Finally it was concluded that different S-1 and rod denaturation profiles found with catfish and tilapia myofibrils were generated by the different structural stability of myosin filaments. Myosin filaments in catfish myofibrils were less rigid than ones in tilapia myofibrils.

Discussion

Myosin plays the most important role in processing of muscle-based product, such as thermal gel from Surimi. We proposed that native myosin content is a useful and sensitive biochemical index for the evaluation of the quality of Surimi [15]. The same principle could be applied to other muscle-based raw materials such as fish itself, fillet, and mince in chilled or frozen form. Myosin denaturation surely proceeds in muscle during the storage. Development of the system to access the myosin denaturation in fish meat using biochemical methods would be useful for monitoring, regulating, evaluating, ensuring, and controlling of the quality of fish meat. As the first step, we characterized the myosin denaturation profile of catfish using tilapia as a reference for collecting the basic information.

Tilapia is the fish from which biochemically characterized myosin was purified by Takashi *et al.* [2]. One of the reasons for the success of purification of myosin was its high stability. As catfish is cultured under similar conditions to tilapia, it is reasonable to think that myosin from catfish is as stable as one from tilapia. Isolated myosin and actin-bound myosin (myofibrils) of catfish were almost as stable as corresponding preparations of tilapia. Catfish used in the experiment was a hybrid from *Clarisas macrocephalus* x *Clarias gariepinus*. No information on the stability of myosin of original catfish species is available at present. The habitat conditions for the original catfish species are the same as the hybrid one, so myosin in the original catfish species might be similarly stable when the principle that water temperature is the determinant factor for the thermal stability of myosin is accepted. The interesting finding was made with Chinese freshwater fish silver carp that silver carp expresses very stable myosin in summer season, and very unstable one in winter. Reported inactivation rate of summer type silver carp myosin at 30 ºC was 5.2 x 10^{-5} sec^{-1} [16], which is similar to that of catfish myosin (16.7 x 10^{-5} sec^{-1}). As year-round change in water temperature for catfish culture ponds in Thailand is not large, such stable myosin seems expressed year-round. Thermal inactivation rate at 30 ºC for marine fish captured in Thailand, croaker and threadfin bream were reported to be 525 x 10^{-5} sec^{-1}, and 647 x 10^{-5} sec^{-1}, respectively [17]. Catfish myosin was roughly 30-38 times stable than these marine fish myosin. Comparison of the inactivation rates of myofibrils between at 0.1 M and 2.0 M provided the stabilizing extent achieved by F-actin binding. The magnitude calculated with catfish was about 100 times, which was the same as tilapia and was much greater than those calculated with carp and Alaska pollock myofibrils showing only 10 to 30 times [18].

To characterize catfish myosin denaturation in detail, S-1 and rod denaturation

pattern when heated as myofibrils in 0.1 M NaCl, pH 7.5 was compared with that with tilapia myofibrils. Quick denaturation of rod than S-1 was the pattern of catfish, and tilapia myofibrils showed the opposite pattern. Moreover, slow decrease of rod production found with tilapia myofibrils was accompanied by the generation of aggregates. It is reported that S-1 and rod denaturation profile was fish-species specific [4]. The above conclusion including ours was led based on the heating experiments in 0.1 M NaCl, pH 7.5. As chymotryptic digestion was conducted in a medium containing 0.05 M NaCl, pH 7.0, and 1 mM EDTA, and as rod denaturation was defined as the decrease of rod production and monomeric rod, the method is detecting structural change of myosin filaments upon heating. The most probable site exposed upon heating would be HMM/LMM junction. It is probable that myosin filament undergoes structural change differently when the rigidity of the filament is different. To examine the possibility, S-1 and rod denaturation in myofibrils was studied by changing pH for the heating because pH changes the rigidity of myosin filaments, i.e., myosin forms rigid and strong filament at acidic conditions, and oppositely forms fragile and weak filaments at alkaline pH. Apparent S-1 and rod denaturation pattern was significantly affected by pH for heating. S-1 denaturation was not affected so seriously by the change in pH from pH 6 to 9, but rod denaturation was significantly affected by the change. As expected, rapid decrease of rod production was a common event at alkaline pH, where less rigid filament structure is suggested. A very slow decrease of rod production at acidic pH was explained by the rigid filaments there, i.e., rigid filament at acidic range was resistant to the thermal treatment and penetration by chymotrypsin within myosin filaments is hard to occur. Relatively quick denaturation of rod at pH 9 and slow denaturation of rod at pH 6 were the common pattern for both species of fish. S-1 and rod denaturation pattern was dependent on the pH for heating. It has been proposed

that S-1 and rod denaturation pattern is fish species-specific [4], which was concluded from the experimental results conducted at pH 7.5. We added a modified proposal that S-1 and rod denaturation pattern is also affected by the factors that affects myosin filament structure, pH in this paper. The pH that gave the characteristic patterns for catfish and tilapia was accidentally pH 7.5, the pH usually used for suspending myofibrils. Catfish myofibrils required the decrease of pH by 1 unit to show a similar pattern to tilapia myofibrils. Fish species-specific filament forming ability of myosin was studied by Matsuura *et al.* [11]. Among the myosin studied, tilapia myosin forms very fragile and short filaments. However, rigidity of myosin filament in tilapia myofibrils was not fragile because myosin in myofibrils was selectively cleaved into S-1 and rod without additional cleavage [19]. We confirmed the fact in this study that tilapia myosin filament was more rigid than catfish myosin filaments. We confirmed it by studying the susceptibility of HMM/LMM junction to chymotrypsin in filamentous myosin at varied pH. The site in tilapia myofibrils was more resistant to increase in pH and HMM/LMM junction was well protected even at pH 8. However, the site was easily exposed by raising pH, and the reduction of pH to 7 was needed to protect the site for catfish. It was concluded pH is a very important factor in altering S-1/rod denaturation pattern of myosin. Indeed catfish myosin is as stable as tilapia as judged by the ATPase inactivation rate, its filaments were less rigid than tilapia.

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Legends of figures

Fig. 1. SDS-PAGE patterns of myofibrils and myosin prepared from catfish and tilapia dorsal muscle. (a) and (b) are myofibrils and myosin, respectively. (c) and (t) are samples from catfish and tilapia. MHC, Act, and TM are myosin heavy chain, actin, and tropomyosin, respectively. LC with numbers are light chain components.

Fig. 2. Thermal inactivation profiles of myofibrils and myosin of catfish and tilapia. Myofibrils (a) suspended in 0.1 M NaCl, 20 mM Tris-HCl (pH 7.5) were heated at 40ºC. Myosin (b) dissolved in 0.5 M NaCl, 20 mM Tris-HCl (pH 7.5) was heated at 30ºC. Samples of catfish (circles) and tilapia (squares) were used. Ca^{2+}-ATPase was assayed at 25ºC in 0.5 M KCl, 20 mM Tris-maleate (pH 7.0), 5 mM $CaCl_2$, and 1 mM ATP.

Fig. 3. Temperature dependent thermal inactivation rates of myofibrils of catfish and tilapia. Myofibrils at 0.1 M NaCl (closed symbols) and at 2 M NaCl (open symbols) were heated at various temperatures and the inactivation rates as measured in Fig. 2 were calculated. Catfish (circles) and tilapia (squares) were analyzed.

Fig. 4. Changes in SDS-PAGE patterns of chymotryptic digest of myofibrils upon heating. Myofibrils of catfish (a) and tilapia (b) suspended in 0.1 M NaCl (pH 7.5) were heated at 40ºC, and digested at 20ºC for 60 min in 0.05 M NaCl, 20 mM Tris-maleate (pH 7.0), 1 mM EDTA by using 1/ 200 (w/w, for catfish) or 1/400 (w/w, for tilapia) of chymotrypsin. The digest (d) and the supernatant at 40 % saturated ammonium sulfate fractionated in the presence of 1 mM Mg-ATP (s) were presented. MHC, Act, and TM are the same as in Fig. 1. S-1 and rod are myosin subfragment-1 and rod of myosin, respectively.

Fig. 5. S-1 and rod denaturation in catfish myofibrils as analyzed by chymotryptic digestion. The data in Fig. 4 were used to estimate S-1 (squares) and rod (circles) content in the digest (closed symbols) and rod in the supernatant at 40% saturated ammonium sulfate (open symbols). Ca^{-2+}-ATPase was also presented as reference (triangles). (a) and (b) are samples from catfish and tilapia.

Fig. 6. S-1 and rod denaturation in myofibrils heated at various pH. Myofibrils suspended in 0.1 M NaCl with various pH as shown in the figures were heated at 40ºC. (a) and (b) are catfish and tilapia myofibrils, respectively. Monomeric S-1 (closed squares), monomeric rod (closed circle) and rod produced (open circle) found in the

digest were estimated. The conditions for the digestion and the estimation of the fragments were done as in Fig. 5. The same symbols as in Fig. 5 were also used.

Fig. 7. Comparison of digestion profiles of catfish and tilapia myofibrils at pH 8. Myofibrils of catfish (a) and tilapia (b) suspended in 0.05 M NaCl, 20 mM Tris-HCl (pH 8.0), 1 mM EDTA were digested at 20ºC by using 1/ 200 (w/w, for catfish) or 1/400 (w/w, for tilapia) of chymotrypsin. Abbreviations were the same as in Fig. 4.

Fig. 1

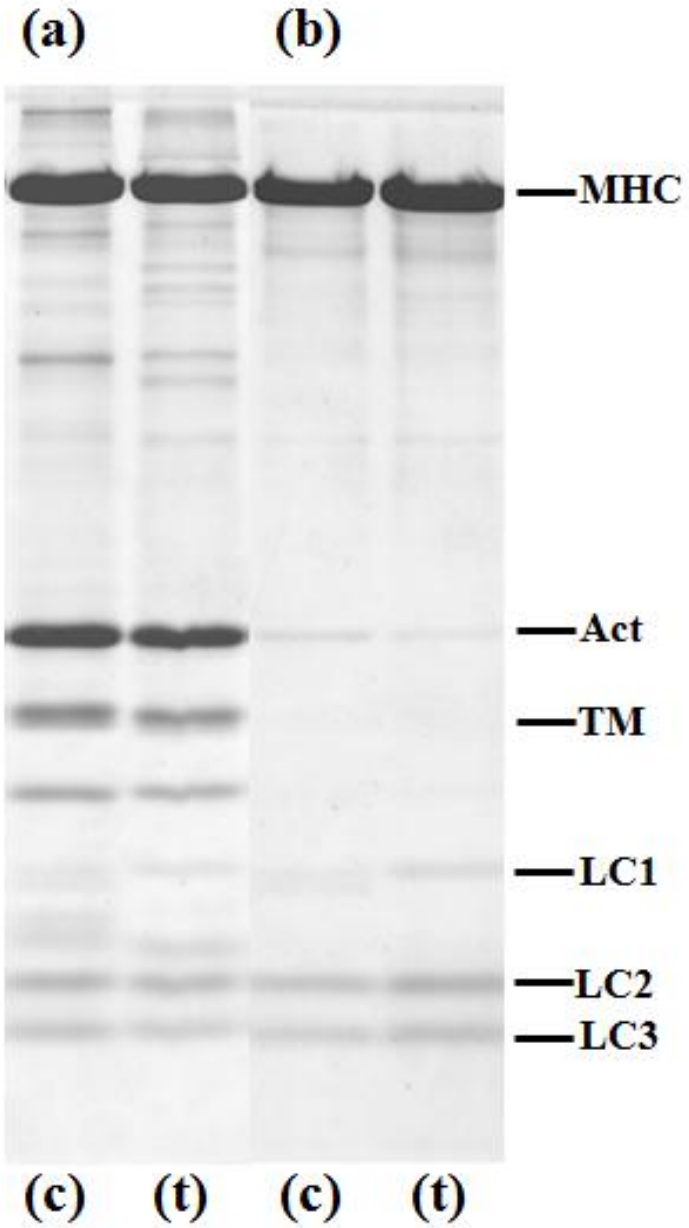


Fig. 2

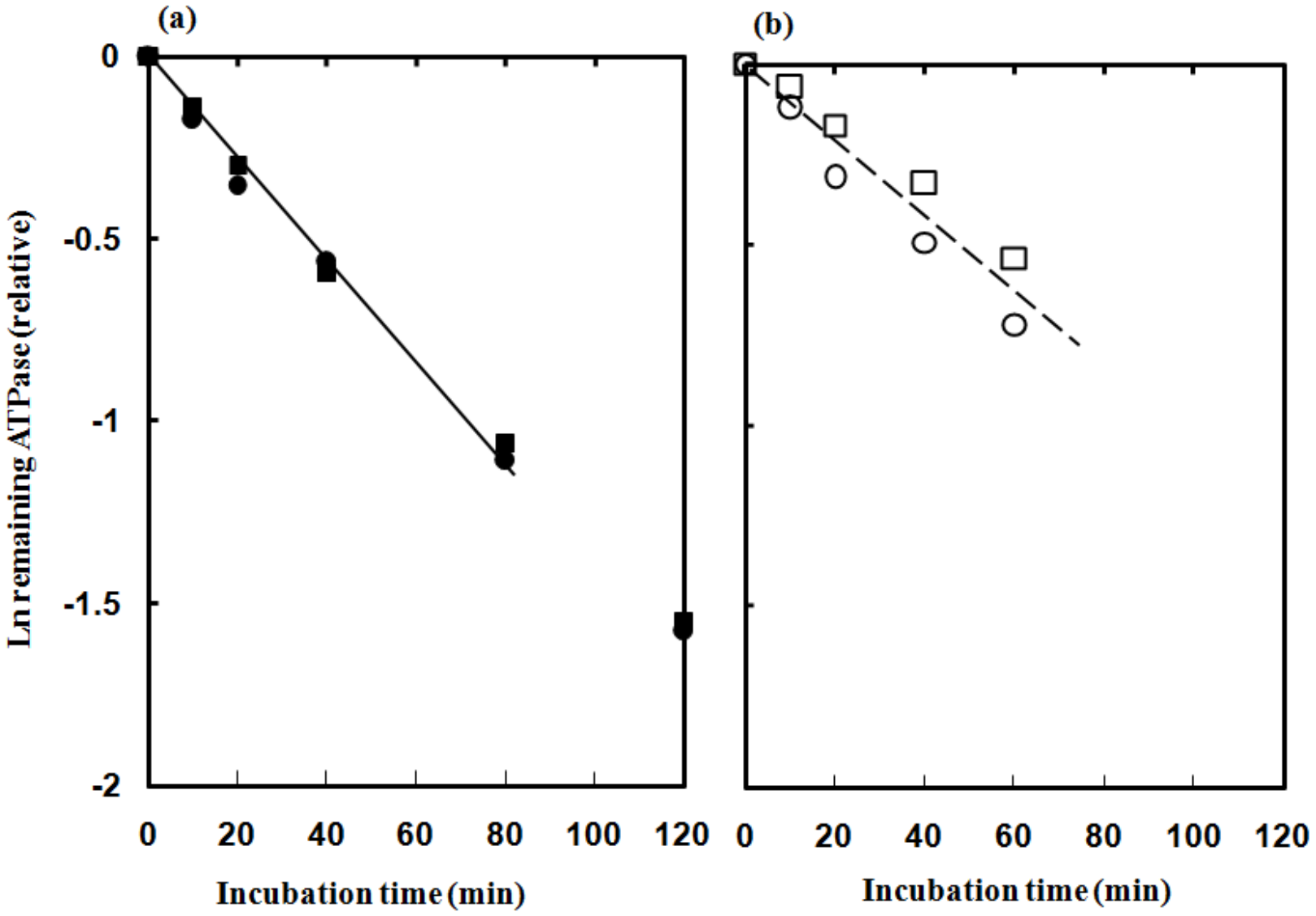

(a)
(b)
0
-0.5
-1
-1.5
-2
Ln remaining ATPase (relative)
0
20
40
60
80
100
120
Incubation time (min)
Incubation time (min)

Fig. 3

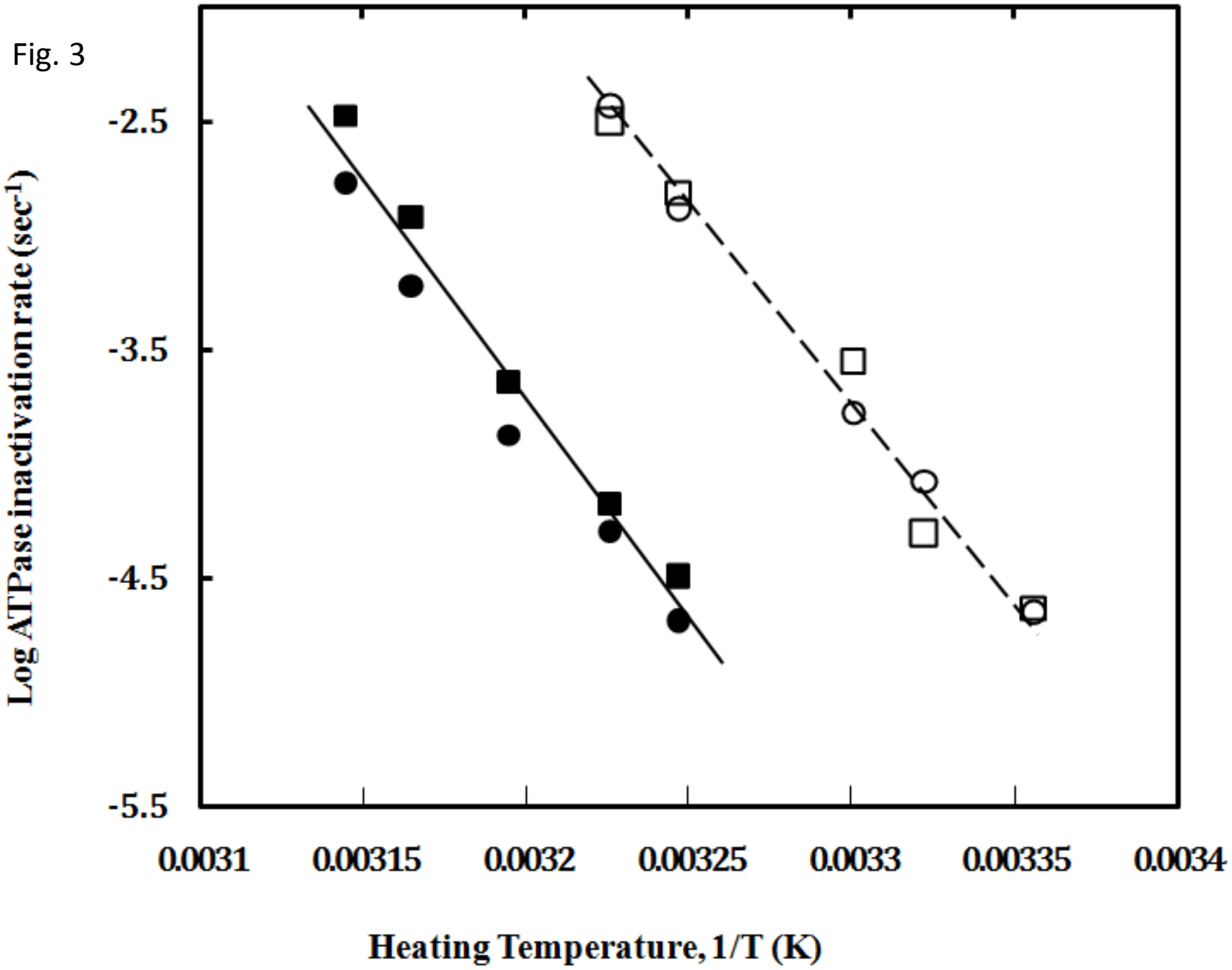

-2.5
-3.5
-4.5
-5.5
Log ATPase inactivation rate (sec-1)
0.0031
0.00315
0.0032
0.00325
0.0033
0.00335
0.0034
Heating Temperature, 1/T (K)

Fig. 4

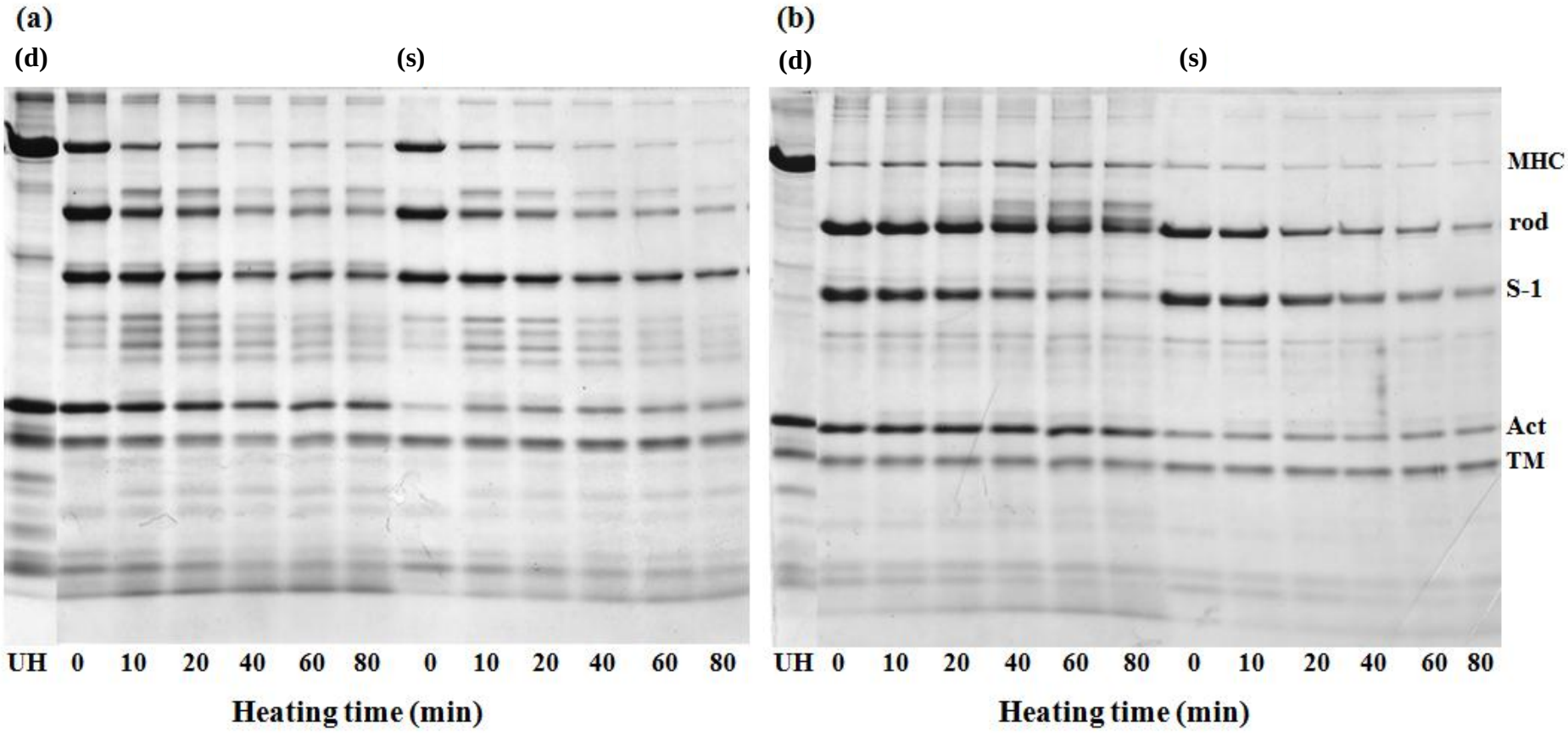

(a)
(d)
(s)
UH 0 10 20 40 60 80 0 10 20 40 60 80
Heating time (min)
(b)
(d)
(s)
MHC
rod
S-1
Act
TM
UH 0 10 20 40 60 80 0 10 20 40 60 80
Heating time (min)

Fig. 5

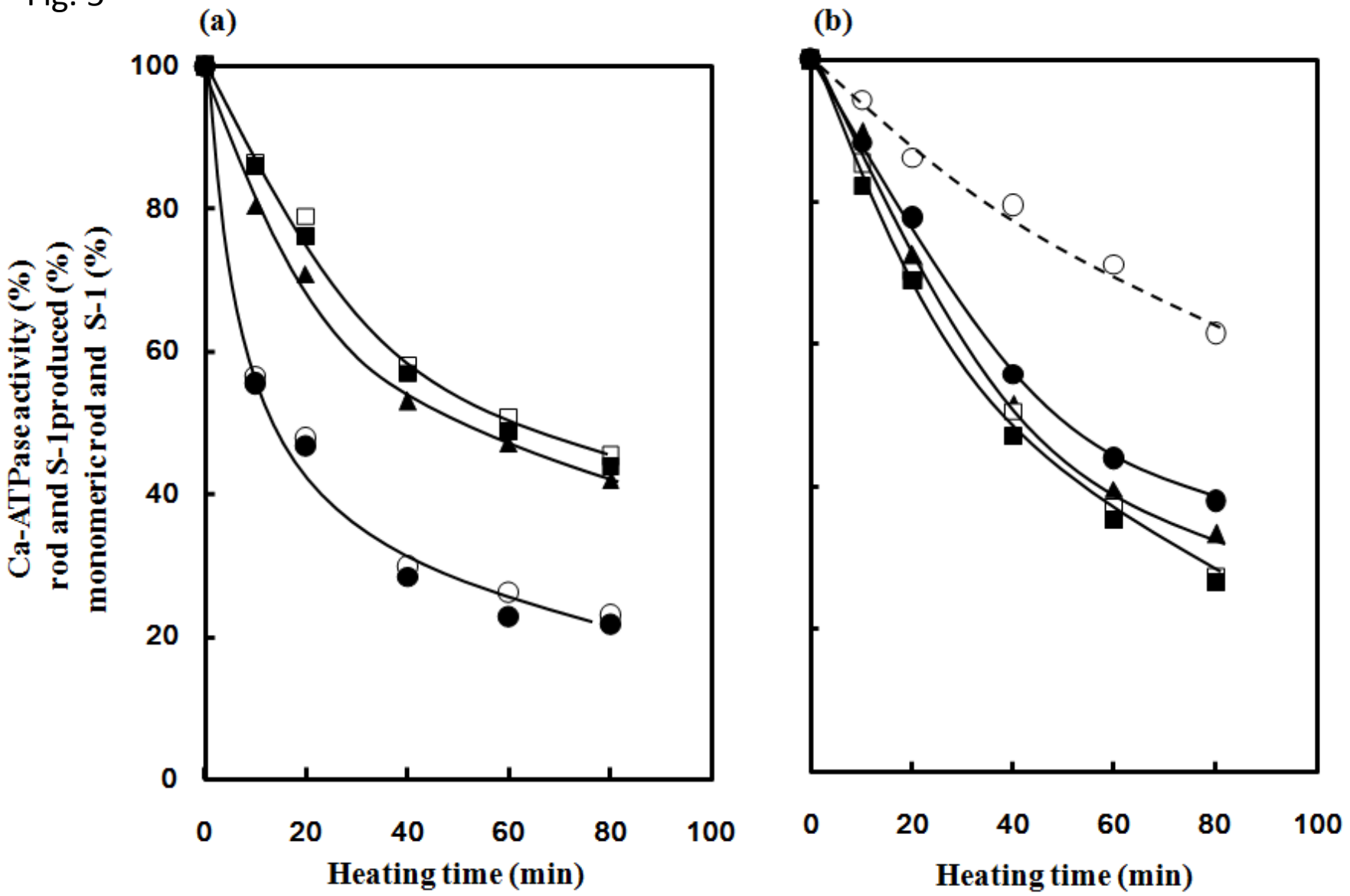

(a)
(b)
Ca-ATPase activity (%)
rod and S-1produced (%)
monomericrod and S-1 (%)
0
20
40
60
80
100
Heating time (min)

Fig. 6

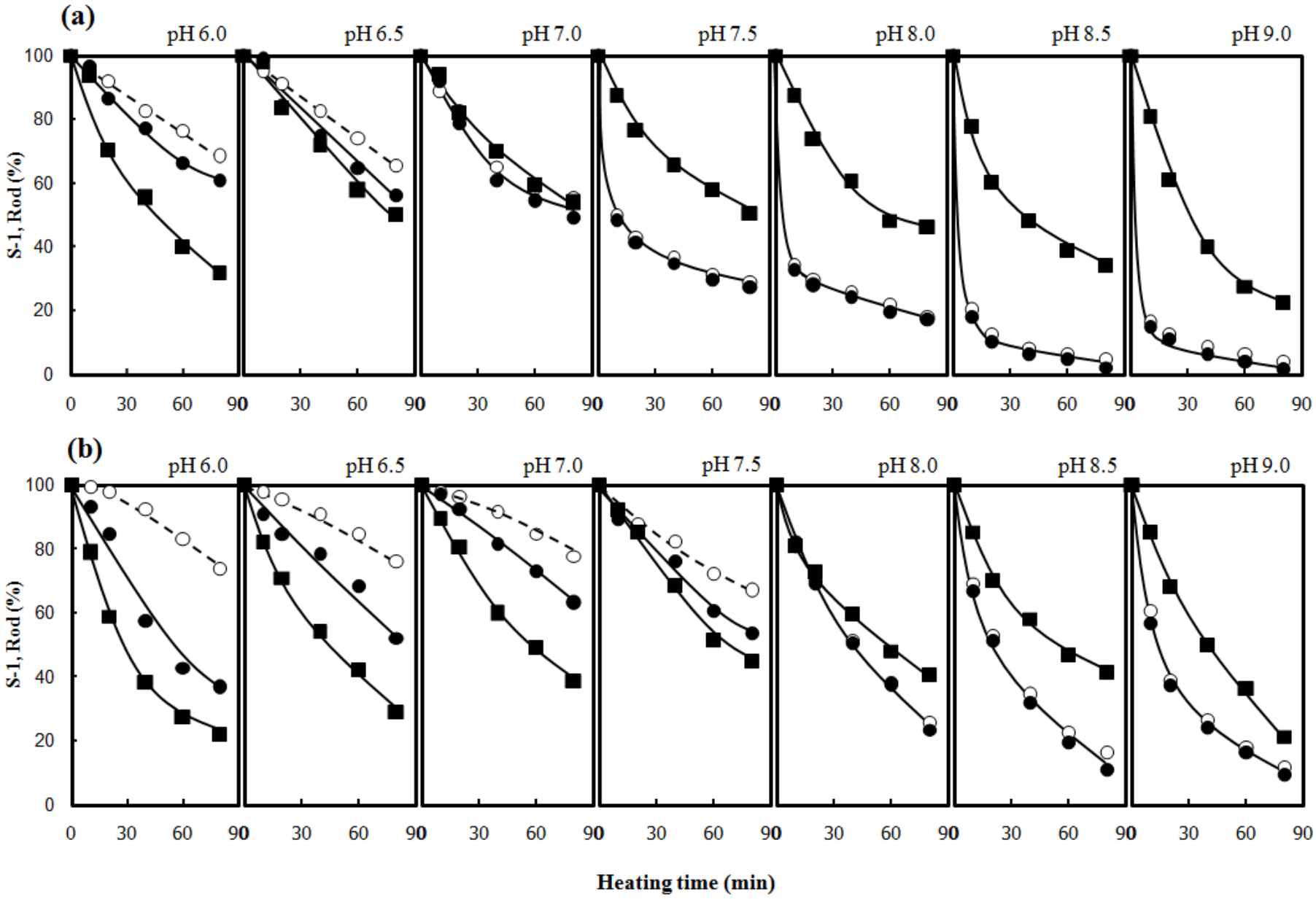
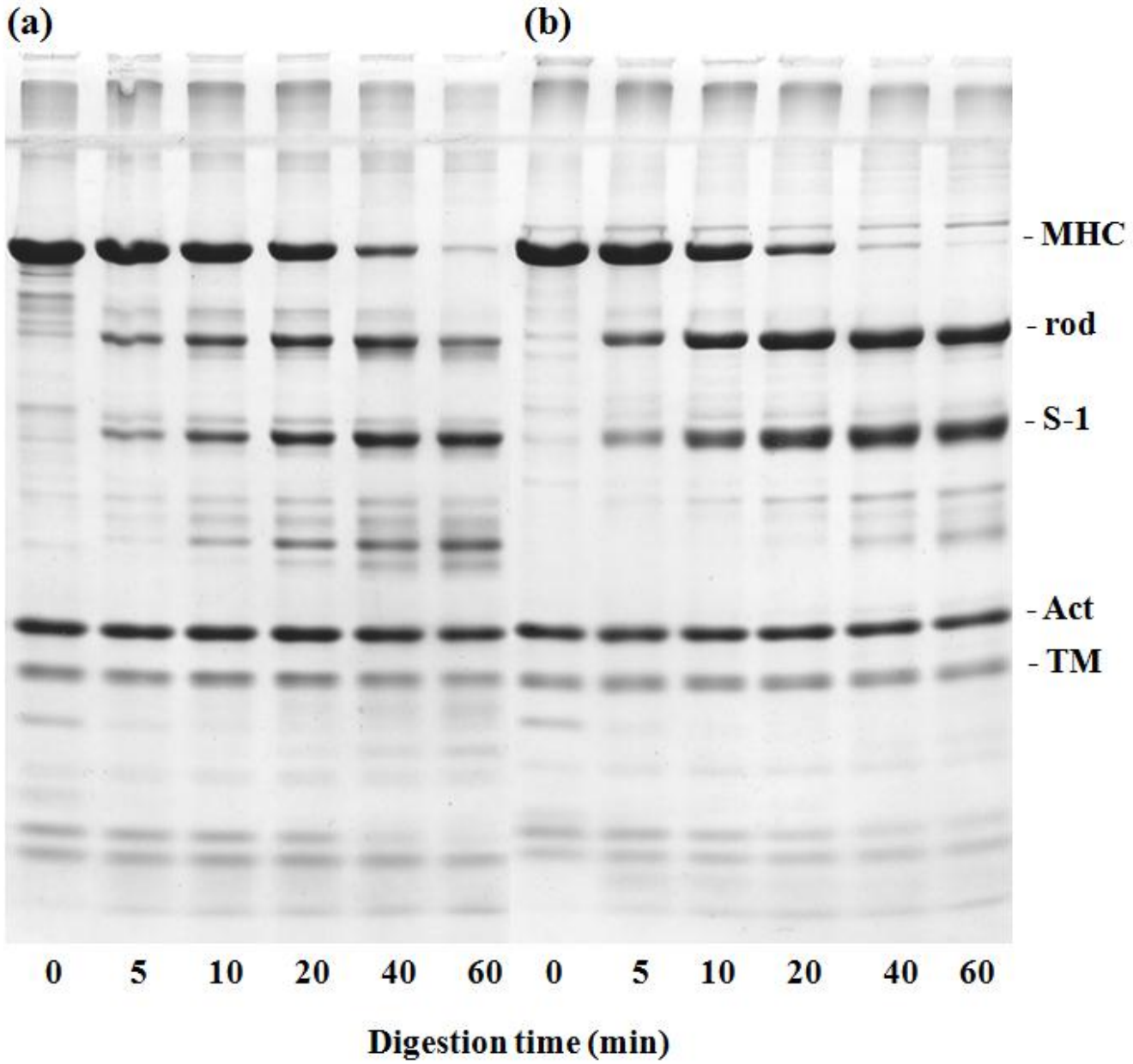

(a)
pH 6.0
pH 6.5
pH 7.0
pH 7.5
pH 8.0
pH 8.5
pH 9.0
S-1, Rod (%)
0
20
40
60
80
100
0
30
60
90
(b)
Heating time (min)

Fig. 7


(a)
(b)
- MHC
- rod
- S-1
- Act
- TM
0 5 10 20 40 60 0 5 10 20 40 60
Digestion time (min)