



Title	Effect of calcium ion on the thermal denaturation of subfragment-1 and rod regions of squid myosin upon the heating of myofibrils
Author(s)	Abe, Kazuyo; 阿部, 一世; Kinoshita, Yasunori et al.
Citation	Fisheries Science, 78(1), 163-168 <a href="https://doi.org/10.1007/s12562-011-0435-7">https://doi.org/10.1007/s12562-011-0435-7</a>
Issue Date	2012-01
Doc URL	<a href="https://hdl.handle.net/2115/48256">https://hdl.handle.net/2115/48256</a>
Rights	© 2012 公益社団法人日本水産学会
Type	journal article
File Information	FS78-1_163-168.pdf



スルメイカ筋原線維の加熱によるミオシン・サブフラグメントー1およびロッド変性に対する  $\text{Ca}^{2+}$  の影響

阿部一世（北大院水）・木下康宣（北海道工技セ）・今野久仁彦（北大院水）

スルメイカ筋原線維の ATPase 失活は  $\text{Ca}^{2+}$  存在下で著しく抑制された。 $\text{Ca}^{2+}$  存在下での ATPase 失活より速い単量体ミオシンの減少は、速いロッドの変性で説明できた。一方、EDTA 中では、単量体ミオシンの減少は Rod 変性より速い S-1 の変性に対応した。同一温度での S-1、Rod 変性速度の比較から、 $\text{Ca}^{2+}$  による安定化は S-1 に限定されると推定した。これは Rod の加熱に伴うヘリックス崩壊に  $\text{Ca}^{2+}$  は影響を与えないことから確認した。それゆえ、イカミオシンの変性様式は  $\text{Ca}^{2+}$  による S-1 の大きな安定化により発現したと結論した。

**Effect of calcium ion on the thermal denaturation of subfragment-1 and rod regions of squid myosin upon heating of myofibrils**

Kazuyo Abe <sup>1</sup>, Yasunori Kinoshita <sup>2</sup>, Kunihiro Konno <sup>1</sup> \*

<sup>1</sup> Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido 041-8611, Japan

<sup>2</sup> Hokkaido Industrial Technology Center, Hakodate, Hokkaido, 041-0801, Japan

\*Corresponding author

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

**Abstract** Thermal inactivation of  $\text{Ca}^{2+}$ -ATPase of squid myofibrils was significantly suppressed in the presence of  $\text{Ca}^{2+}$ . Monomeric myosin content decreased much faster than  $\text{Ca}^{2+}$ -ATPase inactivation in Ca-medium, which was well explained by the fast rod denaturation. In contrast, rod denaturation was slower than S-1 in EDTA-medium. Decrease in monomeric myosin content was explained by faster S-1 denaturation. Comparing the S-1 and rod denaturation rates at the fixed temperature, it was concluded that S-1 denaturation was suppressed by  $\text{Ca}^{2+}$  whereas rod portion was not. Unfolding experiment with isolated myosin rod confirmed no stabilizing effect of  $\text{Ca}^{2+}$  on rod. It was concluded that significant stabilization of S-1 portion by  $\text{Ca}^{2+}$  generated apparently different myosin denaturation pattern in two media.

**Keywords** squid, myosin, thermal denaturation, myofibril, calcium ion

## Introduction

Mantle muscle of squid is distinguished from fish dorsal or mammalian skeletal muscle by its structure. The former shows obliquely striated structure and the latter shows striated structure. Squid muscle is characterized by the myosin-linked muscle contraction regulatory system commonly observed in mollusk muscle such as scallop adductor muscle [1]. In the system, muscle contraction is triggered by the direct binding of  $\text{Ca}^{2+}$  to one of the myosin light chain component (LC) termed as regulatory light chain (RLC) [2, 3], while receptor for  $\text{Ca}^{2+}$  in fish or mammalian muscle is troponin located on F-actin filament [4].

We found unique thermal denaturation profile of squid myofibrils;  $\text{Ca}^{2+}$ -ATPase inactivation was significantly suppressed in the presence of  $\text{Ca}^{2+}$  [5, 6]. Stabilization by  $\text{Ca}^{2+}$  was also detected with myosin subfragment-1 (S-1) [7]. The stabilization by  $\text{Ca}^{2+}$  achieved with myofibrils was much greater than that observed with S-1; stabilizing effect of  $\text{Ca}^{2+}$  on myosin was enhanced through F-actin binding. However,  $\text{Ca}^{2+}$ -ATPase inactivation study does not provide the information on the effect of  $\text{Ca}^{2+}$  on the denaturation of squid myosin tail.

We proposed that chymotryptic digestion of myofibrils detects myosin denaturation at rod portion as well as head region by their decreased production [8]. Myosin denaturation pattern as studied by S-1 and rod denaturation differed from fish species to species. Carp showed a preceded rod denaturation, while slower denaturation of rod than S-1 was the pattern of Alaska Pollock [9]. As the thermal inactivation of squid myofibril  $\text{Ca}^{2+}$ -ATPase was suppressed by  $\text{Ca}^{2+}$ , it is interesting to know whether  $\text{Ca}^{2+}$

stabilizes myosin rod when heated as myofibrils. Moreover, salt-solubility and monomeric myosin content upon heating in the presence and absence of  $\text{Ca}^{2+}$  was studied in relation to S-1 and rod denaturation in two media.

## **Materials and methods**

### Preparation of myofibril

Myofibrils were prepared from the mantle muscle of Japanese common squid *Todarodes pacificus* [5]. Homogenized muscle was washed with 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) containing 1 mM EDTA repeatedly. Inclusion of EDTA was essential for eliminating metallo-type proteolytic activity that degrades myosin selectively [10, 11]. As the incubation of the above myofibrils with  $\text{Ca}^{2+}$  showed practically no myosin degradation as well as in the presence of EDTA, proteolytic activity remaining in the preparation was negligible. Washed myofibril was finally suspended in the above buffer containing no EDTA, and was filtered through two layers of gauze to remove connective tissues, and the filtrate was used as myofibril suspension.

### Thermal denaturation of squid myofibrils

Myofibrils suspended in the above buffer were heated in the presence of either 0.2 mM  $\text{CaCl}_2$  (Ca-medium) or 1 mM EDTA (ED-medium). We confirmed the  $\text{CaCl}_2$  concentration

in Ca-medium was high enough for a full stabilization of myosin [12]. Heating temperature was optionally changed. Myosin denaturation upon heating of myofibrils was studied by monitoring the following indicators; Ca<sup>2+</sup>-ATPase activity, salt solubility, and monomeric myosin content. These were measured as previously reported [13].

S-1 and rod denaturation as studied by chymotryptic digestion of heated myofibrils

Chymotryptic digestion was performed for 60 min at 20°C in a digestion medium of 0.05 M KCl, 20 mM Tris-maleate (pH 7.0) and 1 mM EDTA using 1/400 (w/w) of chymotrypsin over myofibrils. For the digestion, heated myofibrils were diluted with equal volume of 20 mM Tris-maleate (pH 7.0) and 4 volumes of 0.05 M KCl, 20 mM Tris-maleate (pH 7.0) to reduce CaCl<sub>2</sub> or EDTA concentrations contained in the heating medium. EDTA at 1 mM in the digestion medium was high enough to chelate the CaCl<sub>2</sub> coming from Ca-medium (0.025 mM).

To access S-1 and rod denaturation in myosin, amounts of monomeric S-1 and rod produced from heated myofibrils upon chymotryptic digestion were estimated. Monomeric fragments as well as monomeric myosin content were measured by using ammonium sulfate fractionation at 40 % saturation in the presence of 2 mM MgCl<sub>2</sub>-ATP as previously described [13]. The digests and the supernatant at 40% saturation were applied to SDS-PAGE. SDS-PAGE was performed according to Laemmli using 7.5 % polyacrylamide gel [14]. The staining intensity of MHC and S-1 and rod on SDS-PAGE was measured by using Fuji Film Multi Gauge 2.0 system (Fujifilm Co. Tokyo, Japan) after the scanning of

the gel on CanoScan 8000F (Canon Co. Tokyo Japan). All of the changes upon heating of myofibrils were analyzed by applying the first order reaction mechanism [9].

#### Isolation of squid myosin rod

Rod was isolated from the chymotryptic digest of squid myofibrils conducted in the presence of 1 mM EDTA as above. Rod in the digest was purified by using ammonium sulfate fractionation as previously described [15]. Myosin rod was finally dissolved in 0.5 M KCl, 20 mM Tris-HCl (pH 7.5).

#### Unfolding study of myosin rod

Unfolding of squid myosin rod dissolved in 0.5 M KCl, 20 mM Tris-HCl (pH 7.5) with either 1 mM CaCl<sub>2</sub> or EDTA was measured on JASCO spectropolarimeter J 725 (JASCO, Tokyo) [16, 17] using 1mm light path cell. The solution was heated at the raising rate of 1°C/min and the ellipticity at 222 nm upon raising temperature at the rate of 1°C/min was recorded. The ellipticity at 10 and 70°C were assumed to be  $\alpha$ -helix content of 100 and 0%, respectively.

## Results

Changes in Ca<sup>2+</sup>-ATPase, salt solubility and monomeric myosin content upon heating of

myofibrils in Ca-, and ED-media.

Myosin denaturation in the presence of either  $\text{Ca}^{2+}$  or EDTA was compared by measuring salt solubility and monomeric myosin content as well as  $\text{Ca}^{2+}$ -ATPase. Due to large difference in the inactivation rates between two incubation media, accurate measurement of these changes at the fixed temperature was practically impossible. Alternatively, heating temperature was chosen so as to give a similar ATPase inactivation rates in two media; i.e.  $40^{\circ}\text{C}$  and  $33^{\circ}\text{C}$  for Ca-, and ED-media; respectively. A half inactivation of ATPase inactivation was in 20 min in Ca-medium and 30 min in ED-medium. When heated in Ca-medium (Fig. 1a), salt solubility and monomeric myosin content decreased very quickly, roughly 5 times faster than ATPase inactivation. The result indicated that myosin formed aggregates before losing the ATPase activity and that salt-soluble myosin was all monomeric. On the other hand, when heated in ED-medium (Fig. 1b), salt solubility decreased at the same rate as ATPase inactivation and monomeric myosin content decreased slightly faster (1.4 times) than the ATPase inactivation. The results indicated that inactivation led a loss of salt-solubility. It was demonstrated that  $\text{Ca}^{2+}$  in the heating medium changed the myosin denaturation pattern significantly.

S-1 and rod denaturation in Ca-, and ED-media

We also studied the structural changes of rod portion as well as S-1 upon heating in two media. Squid myofibrils heated in Ca- and ED-media were digested so as to convert myosin

into S-1 and rod. SDS-PAGE patterns of the digests obtained are presented in Fig. 2a-(1) and 2a-(2), respectively. S-1 and rod were consisted of several bands suggesting a mixture of several species with different sizes. A quick decrease of rod production relative to S-1 was characteristic pattern in Ca-medium (Fig. 2a-(1)). A gradual increase of the bands migrating above actin band became noticeable with duration in Ca-medium. On the other hand, slower decrease of rod production than S-1 characterized the change in ED-medium (Fig. 2a-(2)). We further studied whether the S-1 and rod in the digests are monomer or aggregated by using salting-out technique. The supernatant at 40% saturation were referred as monomeric fragments sedimenting aggregated ones as pellets [9, 14]. The components in the supernatant were again analyzed on SDS-PAGE (Fig. 2b). S-1 and rod produced irrespective of their amounts were almost completely recovered in the supernatant in two media. Moreover, fragments above actin found in Ca-medium were also recovered in the supernatant. It was thus concluded that the fragments produced, S-1, rod, and other fragments, from heated myofibrils were all monomeric.

Amount of monomeric S-1 and rod in the supernatant appeared in Fig. 2b was estimated on densitometer. The decrease was analyzed by assuming the first order reaction mechanism (Fig. 3). Decrease of rod production proceeded much faster than that of S-1 when heated in Ca-medium and quick loss of monomeric myosin was explained by myosin denaturation at rod portion. Rod denaturation rate relative to S-1 was 4.7 times. ATPase inactivation was well explained by the S-1 content. Oppositely, monomeric rod decrease was slower than S-1 decrease in ED-medium. Rod denaturation rate relative to S-1 was 0.3 times. Being different from in Ca-medium, decrease in S-1 content was slightly faster than

ATPase inactivation indicating that S-1 portion underwent structural change without damaging the active site. A little faster decrease of monomeric myosin content than ATPase inactivation in ED-medium was well correlated with monomeric S-1 content decrease. A little faster decrease of monomer myosin than ATPase was reported with Alaska pollack myofibrils [14]. Myosin underwent damage at its S-1 region but not at active site, which led aggregate formation when heated in ED-medium.

We compared S-1 and rod denaturation pattern of squid myofibrils in two media at different and fixed temperatures. We wondered whether the denaturation profile is affected by heating temperatures from 30 to 42 °C. Squid myofibrils was heated at various temperatures in Ca- and ED-media, and rod and S-1 denaturation rates were calculated as above by measuring monomeric fragment contents. The data obtained were analyzed by using Arrhenius plot (Fig. 4). S-1 and rod denaturation rates in Ca- and in ED-media all gave linear relationships with roughly the same slopes. Accordingly, characteristic S-1 and rod denaturation profiles in Ca- and ED-media were commonly observable at all temperatures within the range. By using the graph, denaturation rates of S-1 and rod in two media at the fixed temperature were calculated by extrapolating the lines. The rate of S-1 denaturation at 37°C in ED-medium was too large to measure, but the calculated rate in logarithmic scale was -2.2 indicating that the stabilization of S-1 by  $\text{Ca}^{2+}$  was 48 times. The rate of rod denaturation in ED-medium was also calculated to be -2.8. Thus the rate in ED-medium was about 3 times greater than in Ca-medium. The stabilization magnitude was negligible compared to one obtained with S-1 denaturation.

## Unfolding profiles of squid myosin rod with $\text{Ca}^{2+}$ or EDTA

Rod denaturation when heated as myofibrils was slightly stabilized by  $\text{Ca}^{2+}$ . It is not certain whether the suppression of thermal denaturation of rod portion is achieved by a direct effect of  $\text{Ca}^{2+}$  on rod. To study the stability of rod in Ca- and ED-media, unfolding profiles of isolated myosin rod was compared in the presence of  $\text{Ca}^{2+}$  and EDTA in monomeric form at 0.5 M KCl, 20 mM Tris-HCl (pH 7.5). The conditions for heating of rod were different from ones for myofibrils. As the stabilizing effect of  $\text{Ca}^{2+}$  was detected with isolated myosin S-1[7], stabilizing effect might be detected with rod solution if present. Relative decrease of ellipticity of rod solution at 222 nm was followed upon raising the temperature in two media. The profiles were presented in Fig. 5. There was no difference in the unfolding profile in two media. When the helical content of rod at 10 and 70°C were taken as 100 and 0 %, a half unfolding was observed at around 34°C. Accordingly, stabilization of rod portion of myosin by  $\text{Ca}^{2+}$  when heated as myofibrils was not direct effect.

## Discussion

Squid myosin has very unique property in thermal denaturation process; myosin was stabilized by  $\text{Ca}^{2+}$  as studied by the suppression of ATPase inactivation [5]. The effect of  $\text{Ca}^{2+}$  was found in myofibrils more significantly than in myosin or S-1 [7, 12]. However, effect of  $\text{Ca}^{2+}$  on rod portion has not been studied yet. To analyze rod denaturation in myofibrils, chymotryptic digestion of the myofibrils is useful tool [9]. S-1 and rod band in

the digests of squid myofibrils contained several minor bands, which were probably due to several cleavage sites near S-1/rod junction as proved with scallop myosin [18]. In the present study, all of the bands were combined in the calculation of S-1 and rod content. Rod produced sometimes contains aggregates which were removed by ammonium sulfate fractionation [9]. We noticed that the presence of paramyosin in the digest made the measurement of S-1 content difficult because of its similar mobility to that of S-1 on the gel. Fortunately, paramyosin was completely removed as precipitates at 40 % saturated ammonium sulfate. Thus S-1 content in the supernatant at 40% saturation as monomeric form was measured. Practically all of rod was monomer, so rod denaturation was also studied by estimating monomeric rod.

Relative denaturation rate of rod to S-1 in Ca- and ED-media was different. Relative to S-1 denaturation, quick denaturation of rod in Ca-medium and slow denaturation of rod in ED-medium were the patterns. Quick decrease of rod production was accompanied by the production of LMM-like or subfragment-2-like fragments migrating above actin band as proved with fish myofibrils [8]. The pattern found in Ca-medium was similar to that of carp myofibrils and slow rod denaturation in ED-medium was rather similar to that of pollock myofibrils [9]. S-1 and rod denaturation explained myosin denaturation such as aggregation detected by loss of salt-solubility and monomeric myosin. Myosin aggregation is the fastest event in two media, but the cause for the change differed from each other. A quick denaturation of rod in Ca-medium explained a quick aggregates formation. Myosin aggregation in ED-medium was explained by S-1 denaturation, where rod denaturation rate was smaller than S-1 denaturation. We proposed that S-1 and rod

denaturation pattern was determined by the fish species [19]. However, squid myofibrils gave different pattern dependent on the conditions for heating. Accordingly, the pattern is fish species-specific but is also affected by the presence and absence of  $\text{Ca}^{2+}$  for the case of squid.

The characteristic patterns in two media were commonly observed between 30 – 42°C as proved by measuring temperature dependent denaturation rates. S-1 denaturation rates in ED-medium were always 48 times larger than those in Ca-medium. Rod in Ca-medium was a little stable than in ED-medium, but the extent was only 3 times. We wondered whether such stabilization is detected with isolated rod.  $\text{Ca}^{2+}$  is able to bind to negative charge on rod surface as  $\text{Mg}^{2+}$  [20, 21]. As the  $\text{Mg}^{2+}$  binding affects myosin filament structure,  $\text{Ca}^{2+}$  binding to rod may stabilize rod portion too. However, the isolated squid myosin rod denatured exactly the same manner in two media when destruction of  $\alpha$  helix upon increase in the temperature was the index. A direct effect of  $\text{Ca}^{2+}$  binding to rod was not probable. Probably great stabilization of S-1 portion by  $\text{Ca}^{2+}$  indirectly affected the denaturation of connecting rod region slightly. It is reported that rod denaturation as studied by aggregate formation was affected by connecting S-1, namely rod aggregates was remarkable when myosin was heated, while no aggregate was formed when S-1 and rod mixture was heated [22]. It was concluded that stabilizing effect of  $\text{Ca}^{2+}$  on myosin is restricted to S-1 portion, and stability of rod portion is primarily unaffected by the presence of  $\text{Ca}^{2+}$ .

## References

1. Kendrick-Jones J, Lehman W, Szent-Gyorgyi A G (1970). Regulation in molluscan muscles. *J Mol Biol* 54: 313-326.
2. Konno K (1978) Two calcium regulatory systems in squid muscle. *J Biochem* 84: 1431-1441.
3. Konno K, Arai K, Yoshida M, Watanabe S (1981) Calcium regulation in squid mantle and scallop adductor muscles. *J Biochem* 89: 581-569.
4. Ebashi S, Kodama A, Ebashi F (1968) Troponin. I. Preparation and physiological function. *J. Biochem.* 64: 465-477.
5. Konno K, Yuasa M (1993) Comparative aspect of thermal stability of squid myofibrils. *Comp Biochem Physiol* 105A: 427-430.
6. Konno K (1991) Thermal denaturation of squid myofibrils: Effects of calcium ion and EDTA. *Nippon Suisan Gakkaishi* 57: 2145-2149.
7. Konno K (1991) Complex formation between regulatory and essential light chain on squid mantle myosin subfragment-1 revealed by thermal denaturation method. *J Biochem* 109: 816-821.
8. Konno K, Yamamoto T, Takahashi M, and Kato S (2000) Early structural changes in myosin rod upon heating of carp myofibrils. *J. Agric. Food Chem* 48: 4905-4909.
9. Takahashi M, Yamamoto T, Kato S, and Konno K (2005) Comparative study on thermal denaturation modes of myosin in walleye Pollack and carp myofibrils as affected by salt concentration. *Fish Sci* 71: 662-671.
10. Tajima T, Tamori J, Kanazawa N, Tamiya T, Tsuchiya T (1998) Distribution of myosinase I and myosinase II in tissue of coleoidea. *Fish Sci* 64: 808-811.
11. Konno K, Fukazawa C (1993) Autolysis of squid mantle muscle protein as affected by storage conditions and inhibitors. *J Food Sci* 58: 1198-1202.
12. Ohno T, Kinoshita Y, Konno K (2011) Stabilizing effect of  $Ca^{2+}$  on myosin and myofibrils of squid mantle muscle as affected by heating conditions. *Fish Sci* 77: 425-430.
13. Koseki H, Kato S, and Konno K (1993) Myosin extractability as a sensitive probe for the thermal denaturation of carp myofibrils. *Nippon Suisan Gakkaishi* 59: 515-518.

14. Laemmli U K (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
15. Kato S, Konno K (1993) Isolation of carp myosin rod and its structural stability. *Nippon Suisan Gakkaishi* 59: 539-544.
16. Nakaya M, Kakinuma M, Watabe S, Ooi T (1997) Differential scanning calorimetry and CD Spectrometry of acclimation temperature-associated types of carp light meromyosin. *Biochemistry* 36(30): 9179-9184.
17. Tazawa T, Takahashi M, Konno K (2005) Uniquely stable 40 kDa subfragment-2 in carp myosin. *J Agric Food Chem* 53: 2242-2247.
18. Konno K, Watanabe S (1985) Two different preparations of subfragment-1 from scallop adductor myosin. *J Biochem* 98: 141-148.
19. Takahashi M, Yamamoto T, Kato S, Konno K (2005) Species-specific thermal denaturation pattern of fish myosin when heated as myofibrils as studied by myosin subfragment-1 and rod denaturation rates. *Fish Sci* 71: 405-403.
20. Kato S Konno K (1993) Filament-forming domain of carp dorsal myosin rod. *J Biochem* 113: 43-47.
21. Matsuura M, Konno K, Arai K (1988) Thick filaments of fish myosin and its actin-activated Mg-ATPase activity. *Comp Biochem Physiol* 90B: 803-808.
22. Tazawa T, Kato S, Katoh T, Konno K (2002) Role of neck region in the thermal aggregation of myosin. *J Agric Food Chem* 50: 196-202.

**Figure captions:**

Fig. 1 Change in the ATPase activity, salt solubility and monomeric myosin content upon heating of squid myofibrils. Squid myofibrils with 0.2 mM CaCl<sub>2</sub> (a) and with 1 mM EDTA (b) were heated at 40 and 33°C, respectively. Ca<sup>2+</sup>-ATPase activity (*circles*) was assayed in the medium of 0.5 M KCl, 25 mM Tris-maleate (pH 7.0), 5 mM CaCl<sub>2</sub>, 1 mM ATP at 25°C. Salt solubility (*squares*) was measured as the recovered myosin content in the supernatant by the centrifugation of the myofibrils suspended in 0.5 M KCl, 20 mM Tris-HCl (pH 7.5) and 2 mM MgCl<sub>2</sub>-ATP. Monomeric myosin content (*triangles*) was measured as the myosin content in the supernatant at 40 % saturated ammonium sulfate.

Fig. 2 Change in the S-1 and rod production from the heated squid myofibrils. Squid myofibrils in Ca-medium (a) and in ED-medium (b) were heated at 40 and 33°C, respectively. Myofibrils were digested at 20°C for 60 min in the medium of 0.05 M KCl, 20 mM Tris-maleate (pH 7.0) and 1 mM EDTA by using 1/400 (w/w) chymotrypsin. SDS-PAGE pattern for the digest (1) and the supernatant at 40 % saturated ammonium sulfate (2) were compared. Rod, S-1, Act, LMM, and TM are myosin rod, subfragment-1, actin, light meromyosin, and tropomyosin, respectively.

Fig. 3 Correlation between S-1 and rod denaturation and loss of myosin functions. (a) and (b) are the results obtained with heated myofibrils in Ca-, and ED-media, respectively. Monomeric S-1 (*closed circles*) and monomeric rod (*closed triangles*) were estimated from

the pattern in Fig. 2B. ATPase inactivation (*open circles*) and monomeric myosin content (*open triangles*) were taken from Fig. 1.

Fig. 4 Temperature dependent thermal denaturation of S-1 and rod. Squid myofibrils were heated at various temperatures in Ca-medium (*open symbols*) or ED-medium (*closed symbols*). S-1 (*circles*) and rod (*triangles*) denaturation rates defined as the decrease in monomeric fragments as in Fig. 3 were measured.

Fig. 5 Unfolding profiles of squid myosin rod in media containing Ca and EDTA. Squid myosin rod in 0.5 M KCl, 20 mM Tris-HCl (pH 7.5) was heated by using a linear increasing program at a rate of 1 degree/min from 10 to 70°C. Decrease in the ellipticity at 222 nm was followed as an index of unfolding or helix structure of rod. Relative ellipticities at 10°C and at 70°C were taken as 100 % and 0 %, respectively as an index of helical content. Solid and open symbols denote the heating with 1 mM CaCl<sub>2</sub> and EDTA, respectively.

Fig.1

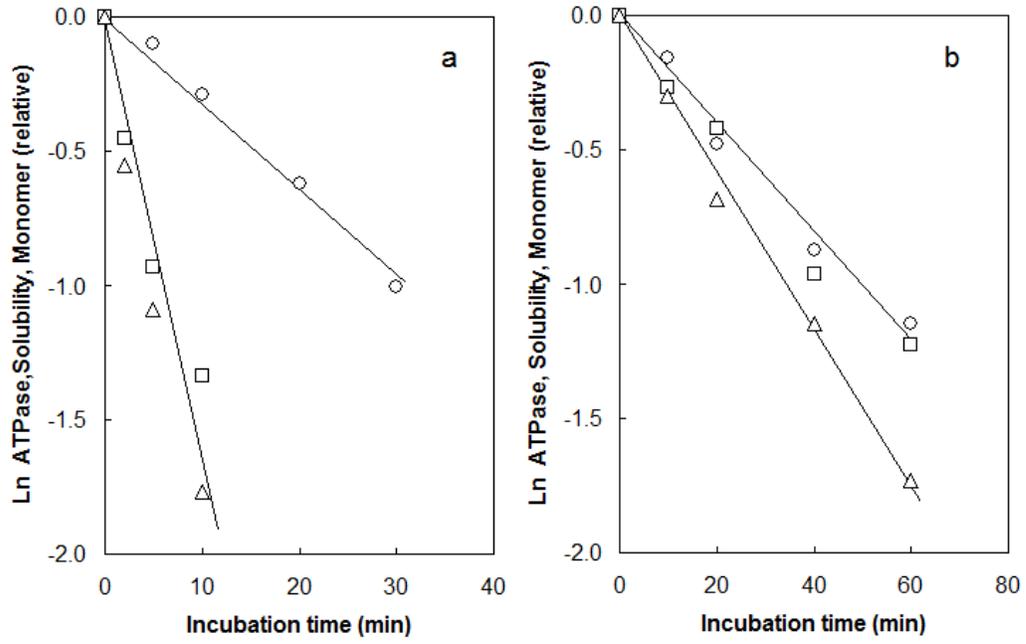


Fig. 2

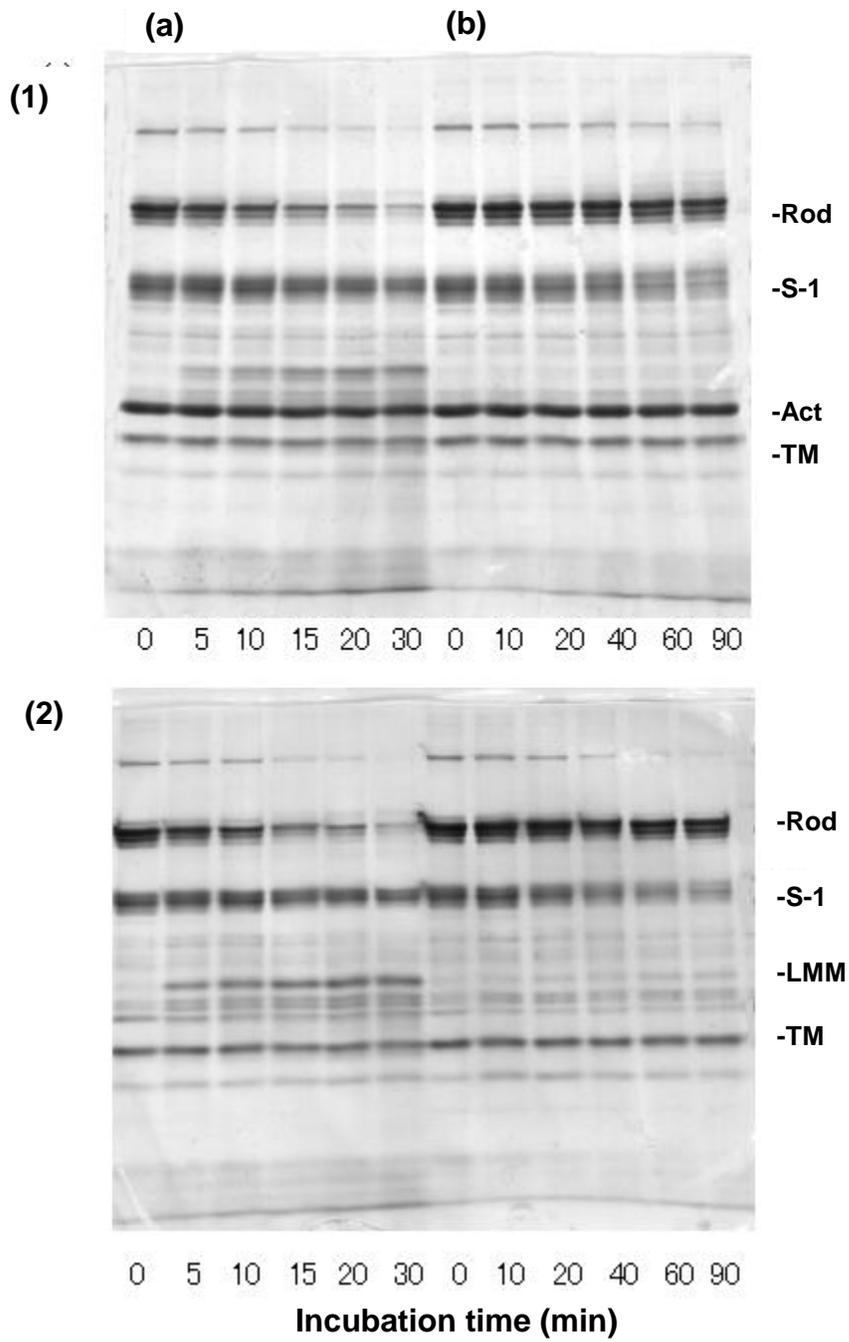


Fig. 3

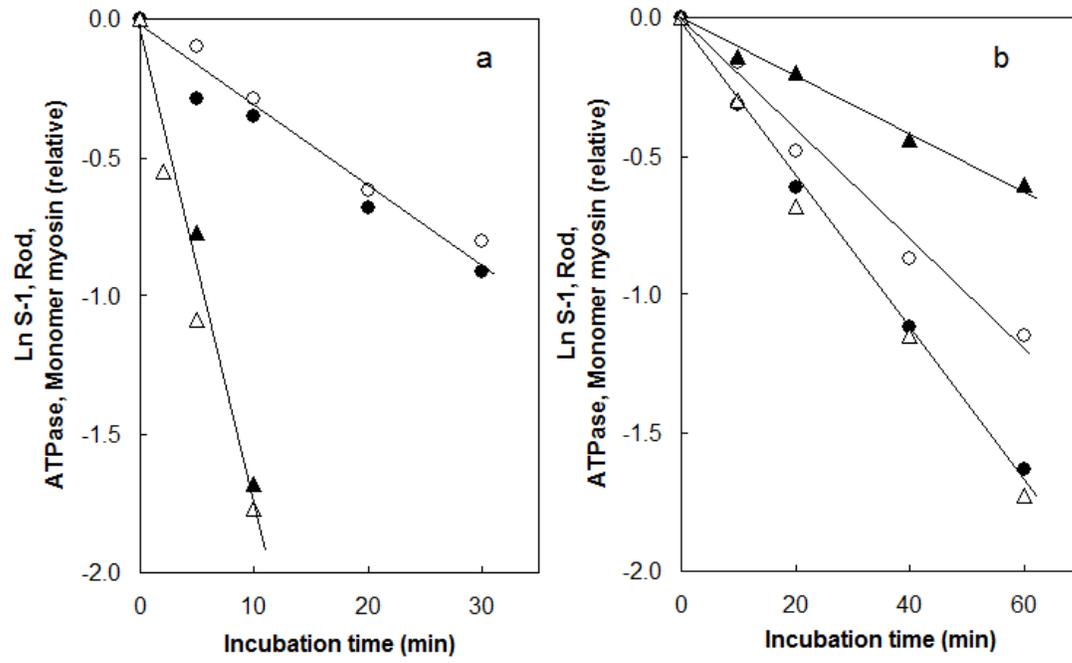


Fig. 4

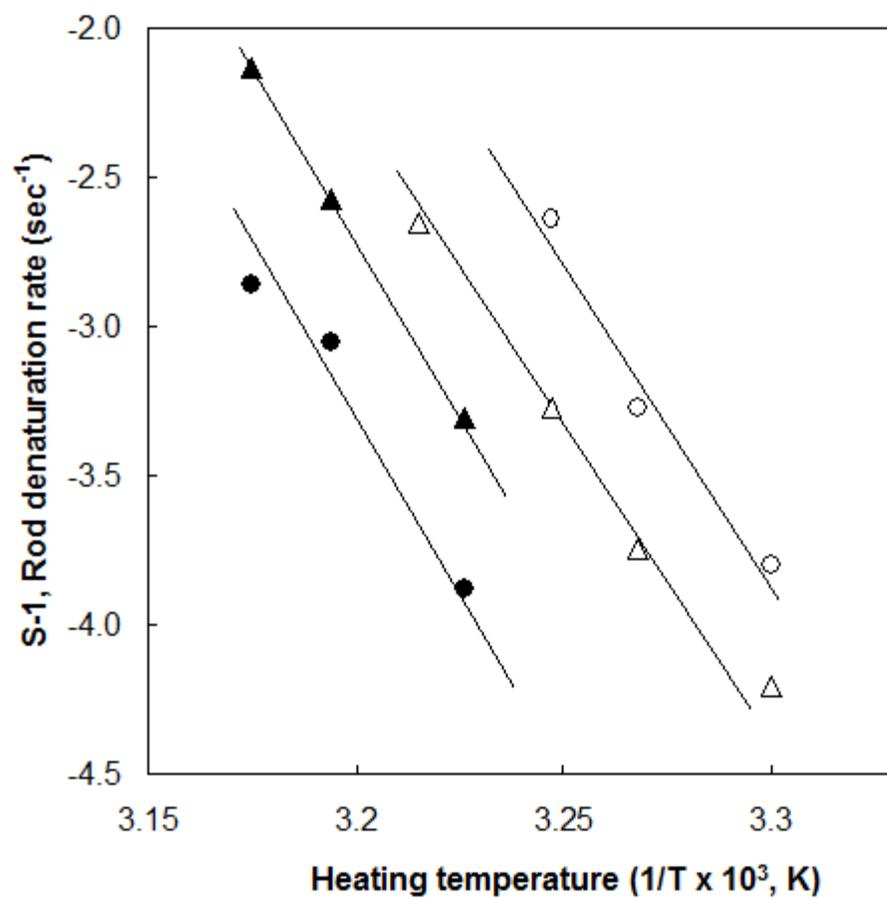


Fig. 5

