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ホタテ閉殻筋筋原線維の加熱によるミオシン変性

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ホタテ筋原線維(Mf)の ATPase 失活は塩濃度上昇で著しく促進されたが、 Ca^{2+} 添加の影響を受けなかった。この特性はイカ Mf と異なった。0.1 M KCl 中の Mf 加熱では ATPase 失活より速く塩溶解性、単量体ミオシンが減少したが、ミオシンのロッド部位の変性で説明できた。パラミオシンを多量に含むホタテ平滑筋 Mf も同じ変性様式を示した。0.5 M KCl 中の Mf 加熱では塩溶解性は維持され、ロッドの緩やかな変性が対応した。

Myosin denaturation of scallop adductor muscle heated as myofibrils

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Abstract Thermal inactivation of Ca^{2+} -ATPase of scallop myofibrils (0.1 M KCl, pH 7.5) was unaffected by the presence of Ca^{2+} . Monomeric myosin and salt-solubility one decreased much faster than Ca^{2+} -ATPase inactivation in both in Ca^{2+} - and EDTA-media, which was well explained by the faster rod denaturation than subfragment-1 (S-1) denaturation. In contrast, when myofibrils were heated at 0.5 M KCl, slow decrease of salt solubility was characteristic, which was also explained by a slow denaturation of rod portion of myosin. Myofibrils from scallop smooth muscle showed the same denaturation pattern as those from adductor muscle. It was demonstrated that myosin of mollusks is not always stabilized by Ca^{2+} .

Keywords scallop, myosin, thermal denaturation, myofibril, calcium ion

Introduction

Myosin-linked Ca^{2+} regulation system was first found in scallop adductor muscle [1], in which muscle contraction is triggered by a direct binding of Ca^{2+} to one of the myosin light chain component (LC) termed as regulatory light chain (RLC) [2, 3]. The system is generally found in mollusk including squid mantle muscle [4]. Unique thermal denaturation profile of myosin was found with squid myofibrils, that is Ca^{2+} -ATPase inactivation was significantly suppressed in the presence of Ca^{2+} [5, 6]. The presence and absence of Ca^{2+} in the heating medium also changed myosin denaturation pattern as studied by denaturation at head (S-1) and rod regions. A quick denaturation of rod with Ca^{2+} , and its slow denaturation with EDTA was the pattern [7]. The difference was explained by the restricted stabilization of head region, but no stabilization at tail region.

As the thermal inactivation of squid myofibril Ca^{2+} -ATPase was suppressed by Ca^{2+} , it is interesting to know whether Ca^{2+} stabilizes myosin of scallop as well. Moreover, thermal denaturation pattern as studied S-1 and rod denaturation is the same as squid myosin. In addition, myosin denaturation in two types of scallop muscle, adductor and smooth muscles is the same because the latter muscle contains very large quantity of paramyosin that forms core of myosin thick filaments [8].

Materials and methods

Preparation of myofibril

Myofibrils were prepared from the adductor muscle of scallop *Patinopectin Ezoensis* [5]. Homogenized muscle was washed with 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) repeatedly and suspended in the same buffer and finally filtered through two layers of gauze and the filtrate was used as myofibril suspension.

Thermal denaturation of scallop myofibrils

Myofibrils suspended in the above buffer were heated in the presence of either 0.2 mM CaCl_2 (Ca-medium) or 1 mM EDTA (EDTA-medium). Heating temperature was optionally changed, that is 39°C or 34°C. Myofibrils dissolved in 0.5 M KCl, 20 mM Tris-HCl (pH 7.5) were also heated. In the case, myofibrils suspended in 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) at 10-12 mg/ml was put into test tubes and KCl concentration was raised to 0.5 M in the individual test tubes to avoid a transfer of viscous dissolved myofibrils at the concentration. After heating, KCl concentration of the samples was diluted with 4 vols of 20 mM Tris-maleate (pH 7.0) to obtain KCl concentration of 0.1 M. At the stage, myofibril concentration was reduced to 1.7-2 mg/ml, which was a suitable concentration for myosin denaturation analysis. Myosin denaturation upon heating of myofibrils was studied by monitoring the following changes; Ca^{2+} -ATPase activity, salt solubility, monomeric myosin content, and the amount of S-1 and rod produced and their monomeric form as previously reported [9]. Chymotryptic digestion was performed for 60 min at 20°C in a digestion medium of 0.1 M KCl, 20 mM Tris-maleate (pH 7.0) and 1 mM EDTA using 1/400 (w/w) of

chymotrypsin over myofibrils. Usually digestion was conducted at 0.05 M KCl. When the dissolved myofibrils in 0.5 M KCl were the sample, reduction of KCl to 0.05 M by dilution was impossible because salt-solubility measurement and monomeric myosin content measurement require myofibril concentration of roughly 2 mg/ml. As the reduction of KCl concentration to 0.1 M was possible, we examined whether the digestion at 0.1 M KCl gives the same pattern as at 0.05 M. Practically, digestion pattern at 0.1 M was the same as one at 0.05 M, and once dissolved myofibrils in 0.5 M KCl also gave the same pattern after diluting to 0.1 M KCl. Dilution method rather than dialysis method was chosen for the easier procedures. Salt-soluble myosin content, monomeric myosin content, and the amount of chymotryptic S-1 and rod together with their monomeric form were all measured as reported earlier [9]. Contents were calculated by using SDS-PAGE and staining intensity measurement of the corresponding bands as described before [7].

Protein concentration was measured by using Biuret method using serum albumin as standard protein.

Results

Changes in Ca^{2+} -ATPase, salt solubility and monomeric myosin content upon heating of myofibrils in Ca-, and EDTA-media

Myofibrils of scallop adductor muscle suspended in 0.1 M KCl, pH 7.5 were heated in the presence of 1 mM CaCl_2 (Ca-medium) and EDTA (EDTA-medium) at 39°C. Myosin denaturation measured by a loss of salt solubility and monomeric myosin content as well as

Ca²⁺-ATPase inactivation were compared between two heating media (Fig. 1). There was no difference in myosin denaturation profiles irrespective of the indicators used for detecting denaturation in two heating media. First of all, Ca²⁺-ATPase inactivation of scallop myofibrils was not affected by the presence of Ca²⁺ in the heating medium at all. A very quick decrease of salt-solubility and monomeric myosin content at the same rates relative to Ca²⁺-ATPase inactivation was the common events found in two media. The rate of solubility loss and monomeric myosin content ($34.5 \times 10^{-4} \text{ sec}^{-1}$) was roughly 19 times greater than ATPase inactivation ($1.8 \times 10^{-4} \text{ sec}^{-1}$). The pattern found with scallop myofibrils was similar to that found with squid myofibrils heated in Ca-medium. However, the difference in the denaturation rates between ATPase inactivation and solubility loss with squid myofibrils was about 5 times [7]. The results clearly indicated that a majority of scallop myosin lost the solubility upon heating before losing the activity. For example, scallop myofibrils heated for 12 min lost the solubility by 85 %, while its remaining activity was as high as 92%.

As the myofibrils were heated at 0.1 M KCl, myosin denaturation rate measured in Fig. 1 was ones in fully stabilized form by F-actin binding. It is reported that increased KCl concentration reduces the protection by F-actin, and the extent of stabilization achieved by F-actin binding at 0.1 M KCl is fish species-specific [9]. Thus, thermal inactivation rates of Ca²⁺-ATPase of myofibrils were measured by changing KCl concentrations. As the accelerated inactivation at high concentrations of KCl was expected, the temperature for heating was lowered to 34°C. Moreover, the highest KCl concentration was set to 0.75 M according to a preliminary experimental result that the rate above the concentration was

practically the same (data not shown). Change in KCl concentration from 0.01 M to 0.1 M practically unaffected the inactivation rate, while the rate increased remarkably between 0.3 M and 0.5 M and the maximal rate was obtained at around 0.75 M (Fig. 2). Taking the rates at 0.1 M ($1.6 \times 10^{-5} \text{ sec}^{-1}$) and at 0.75 M ($368 \times 10^{-5} \text{ sec}^{-1}$) for the calculation of stabilizing effect by F-actin binding, the stabilization upon F-actin binding was calculated to be about 230 times. The magnitude was nearly the same as measured with squid myofibrils in the presence of Ca^{2+} , and was much greater than that in the absence of Ca^{2+} [5]. The magnitude was much greater than that obtained with fish myofibrils [9].

S-1 and rod denaturation found with scallop myofibrils heated in Ca-, and EDTA-media

Myosin denaturation in heated myofibrils was also studied by applying chymotryptic digestion. It is reported that scallop myosin can be cleaved into S-1 and rod even in the presence of Ca^{2+} producing S-1 with a little longer S-1 heavy chain and intact Ca^{2+} binding LC of RLC in its structure [10]. Scallop myosin is also cleavable at a similar site well characterized with fish myosin requiring a removal of Ca^{2+} from the digestion medium [11]. For S-1 and rod denaturation analysis, the digestion was conducted in the presence of EDTA. KCl concentration used was 0.1 M KCl because the patterns at 0.05 M and at 0.1 M were the same. The samples heated at 39°C as in Fig. 1 were digested under the conditions. Although the SDS-PAGE patterns for the digests were not presented, the digest still contained paramyosin that came from parent myofibrils. The contamination by paramyosin made the estimation of S-1 content difficult because it showed a similar mobility to that of

S-1. However, the protein was removed completely as precipitate at 40% saturation of ammonium sulfate. Practically all of S-1 and rod produced were recovered in the supernatant at the saturation of ammonium sulfate (data not shown). The rod and S-1 in the digest was found to be in monomeric forms. There was no difference in the pattern obtained in two heating media showing a quick decrease of rod and slow decrease of S-1. Thus S-1 and rod denaturation was accessed by calculating of monomeric S-1 and rod content (Fig. 3).

The samples heated in Ca-medium were used for the comparison because there was no difference in the conclusion between Ca- and EDTA-media. Decreases in the amounts of S-1 and rod in monomeric form recovered in the supernatant at 40% saturation of ammonium sulfate and various changes of myosin properties as shown in Fig. 1 were compared. General denaturation patterns at 34°C taking 12 hours to follow the denaturation process were practically the same as found at high temperature of 39°C (see Figs 1 and 3). Quick decrease of salt-solubility and monomeric myosin content and preceded rod denaturation than S-1 were characteristic. Ca^{2+} -ATPase inactivation was well explained by the decrease of amount of S-1 reasonably because ATPase active site locates in the part, which was the same conclusion as obtained with fish myofibrils [9]. Loss of salt solubility and monomeric myosin content was well explained by the rod denaturation. It was concluded that myosin that undergoes the cleavage at rod region is present in aggregated form and such aggregates are no longer salt-soluble. As such myosin still retained Ca^{2+} -ATPase activity, the aggregation at tail region was suggested (Fig. 4).

Myosin denaturation for scallop myofibrils were also studied by lowering the temperature to 34°C for heating to study whether S-1 and rod denaturation pattern was altered by the temperature because the pattern with carp myofibrils was affected remarkably [12]. Myofibrils were heated for 12 hours (720 min). The obtained denaturation patterns were the same as those observed at 39°C (Figs. 1 and 3) showing a quick denaturation of rod accompanied by the quick loss of monomeric myosin content and salt solubility (Fig. 4).

Thermal denaturation of scallop myosin when heated as dissolved myofibrils

With carp myofibrils, it is reported that dissolving myofibrils into 0.5 M KCl changed the myosin denaturation pattern significantly [12]. Myosin denaturation pattern of scallop myofibrils upon heating of dissolved myofibrils into 0.5 M KCl was studied (Fig. 4). Because of the accelerated ATPase inactivation at 0.5 M KCl as suggested in Fig. 2, the temperature was lowered to 34°C. It took around 30 min to show the half inactivation of ATPase (Fig. 5). After the termination of the heating, KCl concentration of myofibril solution was reduced to 0.1 M by adding 4 volumes of 20 mM Tris-maleate buffer (pH 7.0). The samples were used for myosin denaturation analysis by using the same indicators used in Figs. 1 and 3. Monomeric myosin content decreased a little faster than Ca^{2+} -ATPase inactivation indicating that myosin formed aggregates before losing the ATPase activity. The same result was obtained with carp myofibrils heated in dissolved form [12]. However, salt-solubility was kept high during the heating. As the measurement was done with heated

myofibrils re-suspended in 0.1 M KCl, myosin filaments formed by the dilution of KCl were easily disassembled upon raising KCl concentration to 0.5 M again. Such salt-solubility was independent on the ATPase inactivation. For example, sample heated for 30 min lost the activity by half, but the 82% of myosin was recovered in the salt soluble fraction. Heating myofibrils in dissolved form suppressed the loss of salt-solubility. S-1 denaturation was much slower than rod denaturation at 0.5 M, which was opposite to the pattern found at 0.1 M. A slow decrease of salt solubility was well correlated to rod denaturation, and ATPase inactivation was explained by S-1 denaturation. As the monomeric myosin content decreased a little faster than S-1 denaturation, myosin formed active aggregates as suggested with carp myosin [13].

Myosin denaturation in myofibrils from smooth muscle of scallop

Scallop adductor muscle is attached by a small piece of different muscle, smooth muscle. The muscle is believed to be involved in the catch mechanism of scallop [8]. The muscle is rich in paramyosin, helical protein with coiled-coil structure [8]. The paramyosin content in smooth muscle is much greater than myosin content. The most abundant protein in thick filaments of smooth muscle is paramyosin not myosin. It forms core of myosin filaments in smooth muscle. Adductor muscle of scallop and squid mantle muscle contain paramyosin, but the amount is less. Thus, it is interesting to study myosin denaturation in myofibrils of smooth muscle of scallop. Preliminary experiment on ATPase inactivation of myosin in the myofibrils gave the information on its stability. It was found that myosin in smooth muscle

was the same as in adductor muscle. Thus, heating was conducted at 39°C. All of the measurements were summarized in Fig. 6. ATPase inactivation rate for the smooth muscle ($2.5 \times 10^{-4} \text{ sec}^{-1}$) was almost identical to that for adductor muscle ($1.8 \times 10^{-4} \text{ sec}^{-1}$). A parallel and quick loss of salt solubility and monomeric myosin content was the characteristic pattern, which was the same as found with adductor muscle myofibrils. The inactivation was explained by the S-1 denaturation. Furthermore, a quick denaturation of rod was explained quick loss of solubility and monomeric myosin content as did with adductor myofibrils. As a conclusion, unique myosin filament structure containing paramyosin core unaffected the myosin denaturation pattern of scallop.

Discussion

Myosin from scallop adductor and squid mantle muscles is very similar in its function, that is both show myosin-linked regulation mediated through Ca^{2+} binding to its RLC. However, binding manner of RLC in scallop and squid was different; washing with EDTA solution detaches RLC of scallop myosin easily even at low temperature, while RLC of squid myosin never be washed away with the same washing procedures [3]. Removal of RLC from squid myosin required treating myosin with high concentration of urea [3]. As squid myosin is strongly stabilized by Ca^{2+} , it is interesting to study whether scallop myosin is similarly stabilized by Ca^{2+} . The results in Fig. 1 clearly denied the possibility. Heating in Ca- and EDTA-media gave the same thermal inactivation of Ca^{2+} -ATPase for scallop myofibrils. The fact clearly showed that the stabilization by Ca^{2+} is not commonly

observable among mollusk myosin. There are two explanations for the same thermal inactivation in two media, namely scallop myosin is kept stable irrespective of the presence of Ca^{2+} or scallop myosin is not stabilized by Ca^{2+} . Magnitude of stabilization of scallop myosin by F-actin binding detected at 0.1 M KCl (about 230 times) was much greater than that for squid myosin in EDTA-medium (40 times) and rather similar to one for squid myosin in Ca-medium (500 times). It was suggested that scallop myosin was kept stable irrespective of the presence or absence of Ca^{2+} or scallop myosin does not require Ca^{2+} for a full protection by F-actin. RLC of scallop myosin in myofibrils is easily detachable upon their heating in EDTA-medium. Thus, RLC in scallop myosin seemed not contribute to the stability of scallop myosin. It seems interesting to know how thermal denaturation of myosin in other mollusks myofibrils is affected by the addition of Ca^{2+} . Studied on the point is now on going.

A fast rod denaturation relative to S-1 was characteristic for scallop myofibrils heated at 0.1 M KCl. We reported that relatively large magnitude of stabilization by F-actin leads to a fast rod denaturation with fish species [9]. A rapid denaturation of rod observed with scallop myofibrils can be explained similarly with a great stabilization extent. We assumed myosin with the same stability at head and rod regions but different magnitude of stabilization by F-actin. Such systems were assumed to be heated under the same conditions. S-1 denaturation rate for myofibrils with low magnitude of stabilization is greater than that with high magnitude of stabilization. On the other hand, rod denaturation proceeds at the same rate for two cases. Consequently, two systems give different S-1 and rod denaturation patterns. Relative rod denaturation rate to S-1 denaturation became greater for the case with

high extent of stabilization. Structural change occurring in myosin rod region as detected by chymotryptic digestion was always accompanied by the loss of salt-solubility. The conclusion was also true for the case of heating of myofibrils in dissolved form (Fig. 5).

Scallop muscle is consisted of two parts. A major one is adductor and a minor one is smooth muscles. The latter contains a large quantity of paramyosin whose content is higher than that of myosin. Paramyosin is believed to construct the core of thick filaments. As the content of paramyosin in adductor and smooth is different and muscle structure is different, striated and smooth, different myosin denaturation pattern was expected. However, the results obtained with smooth muscle myofibrils were exactly the same as obtained with myofibrils of adductor muscle. Therefore it was concluded that paramyosin core in myosin filaments in smooth muscle of scallop did not affect myosin denaturation, especially rod denaturation.

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Figure captions:

Fig. 1 Myosin denaturation in scallop myofibrils heated with Ca^{2+} or EDTA. Myofibrils of scallop adductor muscle suspended in 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) were heated at 39 °C in the presence of 1 mM CaCl_2 (a) or EDTA (b). Ca^{2+} -ATPase inactivation (circles), salt-solubility (squares), and monomeric myosin (triangles) were estimated.

Fig. 2 Effect of KCl concentration on the Ca^{2+} -ATPase inactivation rates of scallop myofibrils in the presence of either Ca^{2+} or EDTA. Myofibrils suspended in various concentrations of KCl buffered with 20 mM Tris-HCl (pH 7.5) were heated at 34 °C in the presence of 1 mM CaCl_2 (open circles) or EDTA (closed circles). Ca^{2+} -ATPase inactivation rate in logarithmic values were used for plotting.

Fig. 3 S-1 and rod denaturation of scallop myosin when heated as myofibrils with Ca^{2+} or EDTA. Myofibrils in 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) were heated at 39 °C in the presence of 1 mM CaCl_2 (open) or EDTA (closed) as in Fig. 1. Myofibrils were digested with chymotrypsin for cleaving myosin into S-1 (circles) and rod (triangles). Changes in were analyzed by assuming the first order reaction.

Fig. 4 Myosin denaturation in scallop myofibrils heated at low temperature. Myofibrils in 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) were heated at 34 °C in the presence of 1 mM CaCl_2 . (a), Ca^{2+} -ATPase inactivation (circles), salt-solubility (squares), and monomeric myosin (triangles) were estimated. (b), S-1 (circles) and rod (triangles) were measured.

Fig. 5 Myosin denaturation in scallop myofibrils when heated at 0.5 M KCl

Myofibrils in 0.5 M KCl, 20 mM Tris-HCl (pH 7.5) were heated at 34 °C. (a), Ca^{2+} -ATPase inactivation (circles), salt-solubility (squares), and monomeric myosin (triangles) were estimated. (b), S-1 (circles) and rod (triangles) were measured.

Fig. 6 Myosin denaturation in scallop smooth muscle myofibrils. Myofibrils prepared from smooth muscle of scallop suspended in 0.1 M KCl, 20 mM Tris-HCl (pH 7.5) were heated at 39 °C. Changes in Y axis included all of the following changes; ATPase inactivation (open circles), salt-solubility (open squares), monomeric myosin (open triangles), amount of monomeric S-1 (closed circles) and rod (closed triangles).

Fig.1

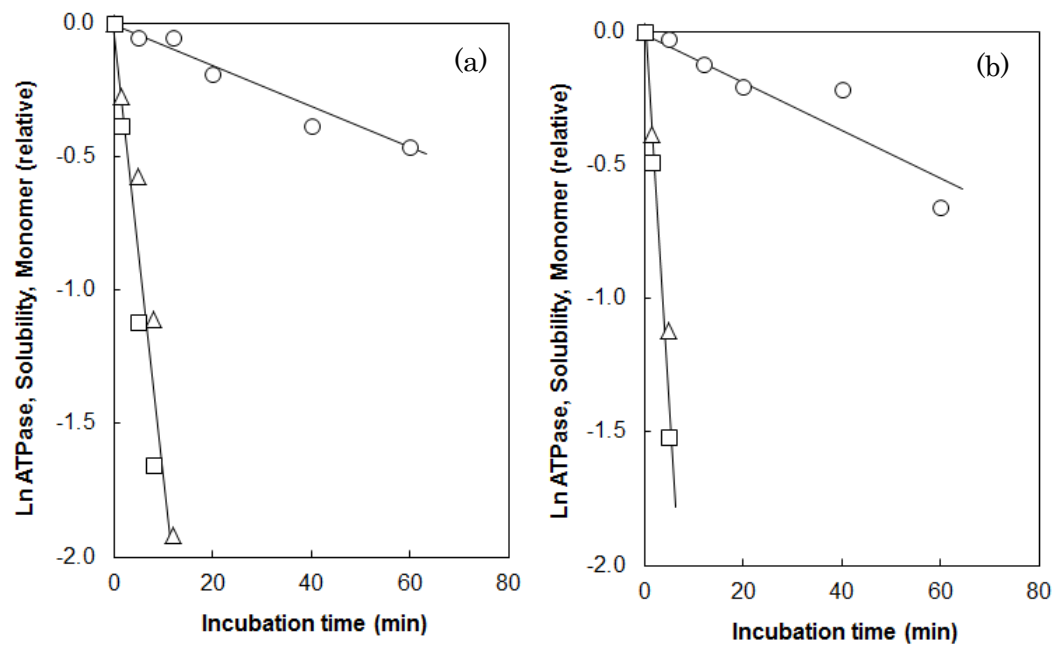


Fig. 2

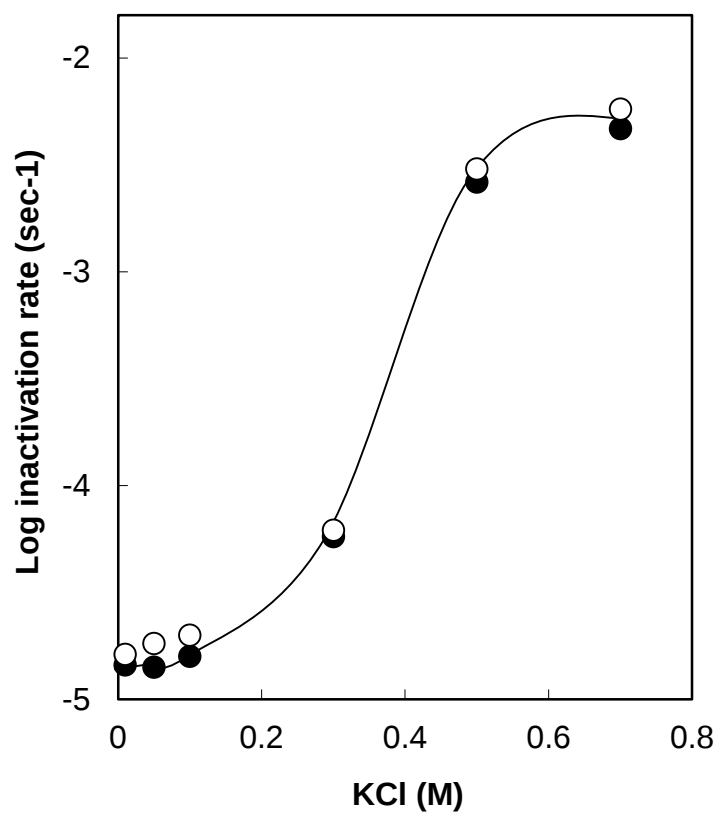


Fig. 3

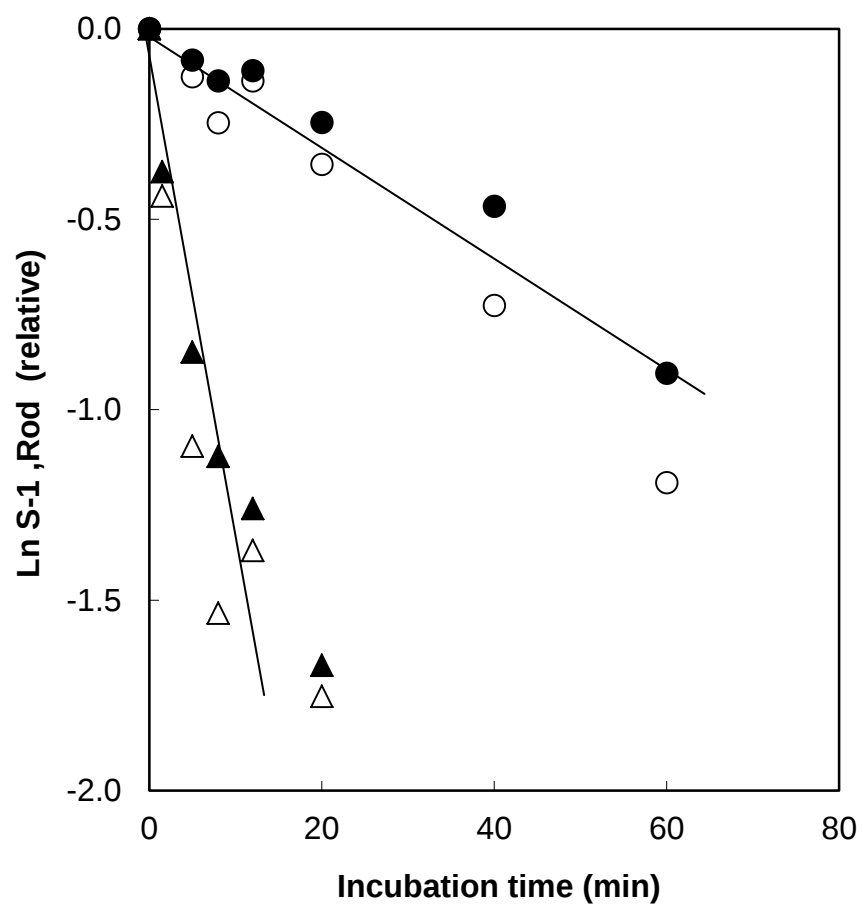


Fig. 4

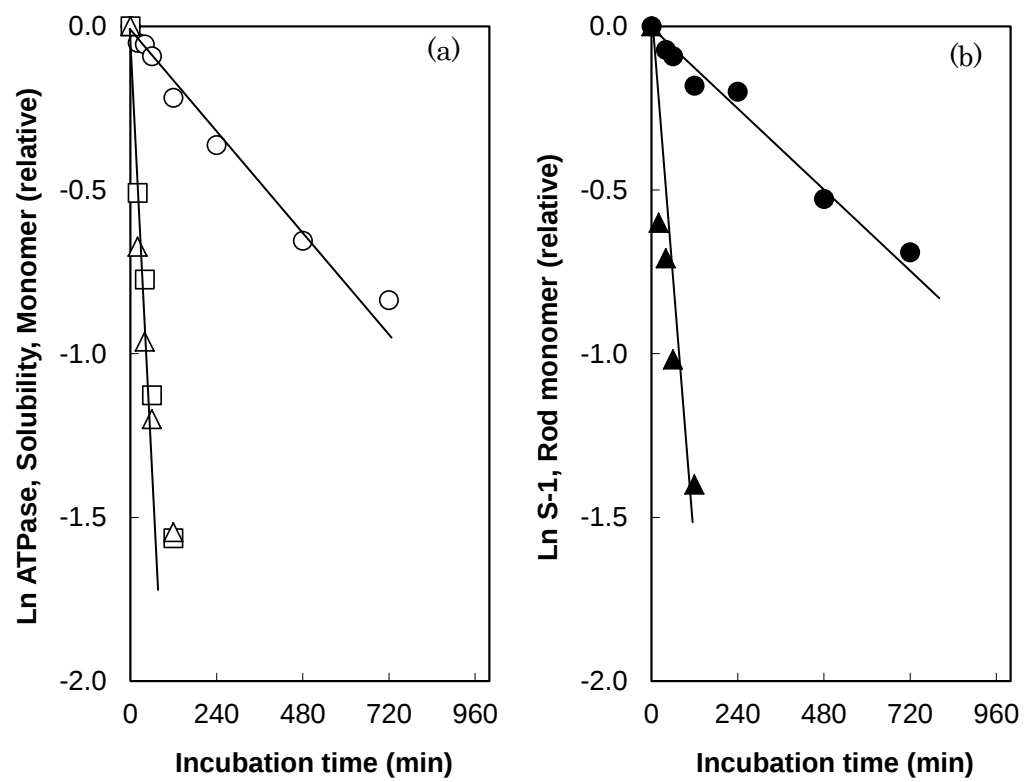


Fig. 5

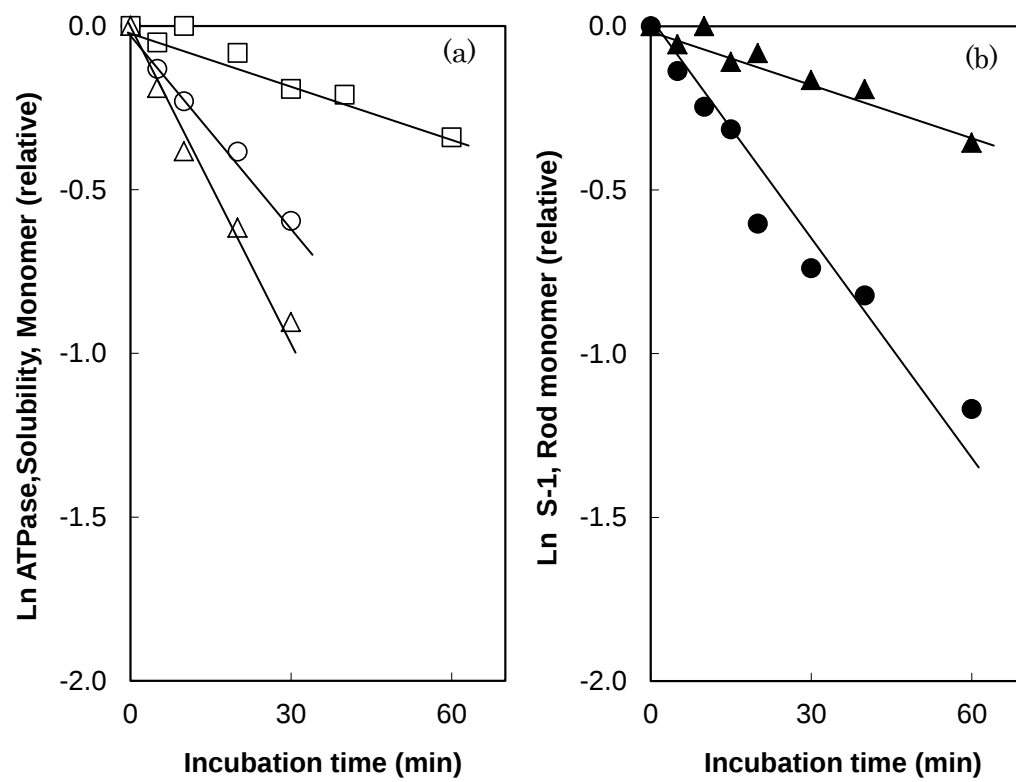


Fig.6

