COMPARATIVE BIOCHEMICAL STUDIES ON THE ATP-MYOSIN B SYSTEM*)**)

By

Kosœak MARUYAMA and Yuji TONOMURA***)

(Received September 14, 1957)

1. Introduction

Since the elegant pioneer works of ENGELHARDT[1], WEBER[2], SZENT-GYÖRGYI[3] and others on the biochemistry of muscle contraction, it has generally been accepted that the ATP-actomyosin system is essential for muscular function (cf. Ref. 4). These studies were done chiefly with mammalian skeletal muscles, of which rabbit muscle attracted special interest.

During the recent several years, the comparative study of the ATP-myosin B (natural actomyosin) system has been conducted in our laboratories with several animals belonging to different phyla. Although some extensive papers were published on the comparative biochemistry of muscle tropomyosin[5-10], no report has been contributed to the similar field of myosin Bs. We might review in the present article the comparative studies of myosin Bs performed mostly in our laboratories.

2. Physicochemical Properties

(1) Ultraviolet absorption spectra

Ultraviolet absorption spectra of myosin Bs from various muscle are nearly the same as shown in Fig. 1; a maximum and a minimum density are observed at 275–278 mμ and 250–255 mμ respectively. Table I shows the extremum extinction coefficients and their ratios. Con-

*) Supported in part by a grant-in-aid given to the Research Group on the "Chemistry of Muscle Contraction" from the Ministry of Education.

**) In this article, the following abbreviations are used; ATP, ADP and AMP = adenosine tri, di and monophosphates, ATPase = adenosinetriphosphatase, EDTA = ethylenediaminetetraacetic acid, P = inorganic orthophosphate.

***) K. M.; Biological Institute, College of General Education, University of Tokyo.
Y. T.; Research Institute for Catalysis, Hokkaido University.

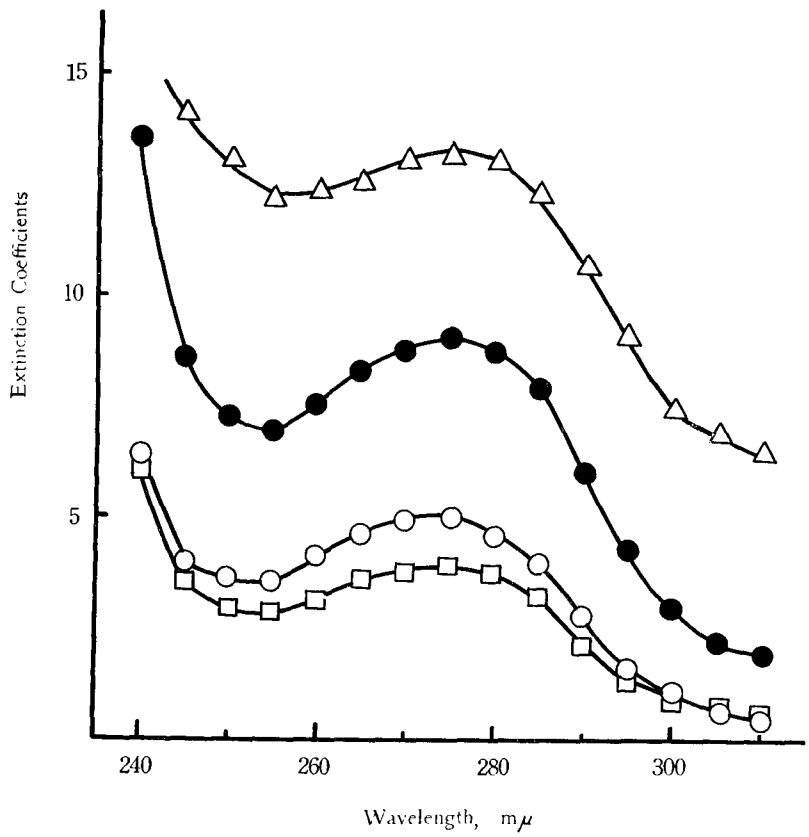

Fig. 1 Ultraviolet absorption spectra of myosin Bs from different sources.
Extinction coefficients on the basis of g. N/L. and 1.0 cm light path. ○, striated portion of adductors of *Meretrix*; □, striated portion of adductors of *Cristraria*; △, thoracic muscle of the honeybee; ●, tail muscle of the crayfish.

siderably high extinction coefficients found in insect myosin B may be partly due to the turbidity caused by some large particles.

Myosin B contains, when conventionally prepared, minor amounts of both nucleotides and ribose nucleic acids; the content of such contaminants is about 1 mole per 10^5 g of myosin B. A high density at 255 mμ of the myosin B spectra from pecten fast adductor is ascribed to a rather high amount of contaminants in this protein[42].

TABLE I. Ultraviolet absorption spectra of myosin B

Animal	Type of muscle	ε_{275}*)	$\varepsilon_{275}/\varepsilon_{255}$	Ref.
Mammals:				
Rabbit	skeletal	3—4	1.3	11
Molluscs:				
Pecten	fast adductor	7	1.2	12
Pecten	slow adductor	4	1.5	12
Cristaria	fast adductor	4	1.3	13
Meretrix	fast adductor	5	1.3	13
Arthropods:				
Honeybee	thoracic	13	1.1	14
Crayfish	tail	9	1.3	15

*) On basis of optical density at 1g. N/L, 1 cm light path in the presence of 0.6 M KCl at room temperature.

(2) Solubility in potassium chloride solution

One of the most characteristic properties of myosin B is its solubility in neutral alkali salts. Rabbit myosin B is completely soluble in more than 0.4 M KCl, whereas easily precipitates from less than 0.3 M. Comparative studies indicated that the solubility in KCl solution is generally much the same, in spite of the great difference in ionic strenght of intracellular media of living muscles from which myosin Bs were extracted.

(3) Salting-out analysis

SNELLMAN and TENOW[16] applied the salting-out technique to the identification of structural proteins of rabbit muscle. According to their results, the range of $(NH_4)_2SO_4$ concentration to precipitate the protein in 0.6 M KCl is: actin (9–20%), actomyosin (28–32%), myosin (33–45%), tropomyosin (45–55%) and nucleotropomyosin (55–64%). However, attention should be paid to tropomyosin A, discovered recently by the LAKI school[7] in smooth muscles of some invertebrates, which precipitates from 33% saturated solution of $(NH_4)_2SO_4$.

Most of myosin Bs prepared by us were analyzed by the salting-out technique (Fig. 2). A larger part of the proteins contained in the myosin Bs from pecten adductor muscles[12], honeybee thoracic muscle and crayfish tail muscle[15] precipitated from 30–35% saturated solution

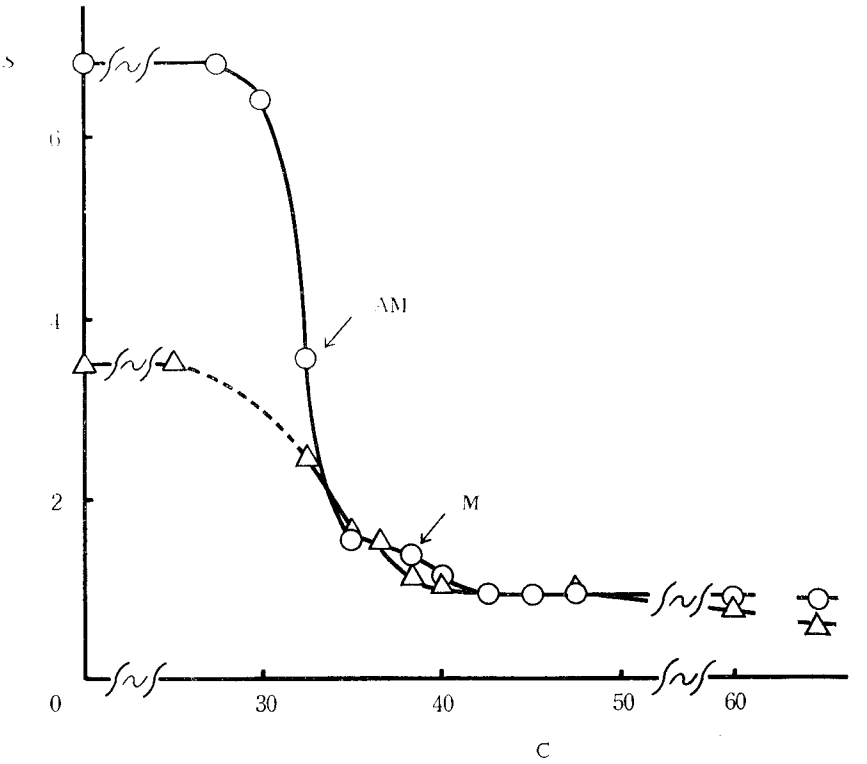

Fig. 2 Salting-out curves of myosin Bs.
S, extinction at 280 mμ (1 g. N/L., 1 cm light path); C, % in volume of saturated $(NH_4)_2SO_4$. ○, the fast adductor of pecten; △, the slow adductor of pecten.

of $(NH_4)_2SO_4$. This fact may indicate that myosin Bs actually consists of (acto) myosin or both of (acto) myosin and tropomyosin A (see p. 61). The only exception was a contractile protein extracted from sea-anemone which precipitated from 35–40% saturated solution of $(NH_4)_2SO_4$[17].

Ultracentrifugal analysis of insect myosin B indicated that there were two main components, one large peak precipitating faster ($s>$ 20 S; very probably actomyosin) and another small one doing slowly ($s=5$ S; very probably myosin)[18,19]. This fact agrees with the results of the salting-out analysis.

3. Superprecipitation and Change of Viscosity

(1) Superprecipitation

The so-called superprecipitation is the most distinguished phenomenon of actomyosin, which has first been discovered by SZENT-GYÖRGYI in 1942 with rabbit myosin B by the addition of ATP. The superprecipitation occurs at rather a limited condition; the best one is: *ca.* 1 mg protein per ml, 0.1 M KCl, pH 7, 20–30°C and *ca.* 1 mM ATP. A typical sort of superprecipitation took place under such condition in myosin Bs from such muscles as tube-feet of the starfish (*Asterias*)[20], adductors of pecten[21], *Cristaria* and *Meretrix*[13], insect thoracic muscle[14] and crayfish tail muscle[15]. The sea-anemone protein was slowly precipitated with ATP[22]. The dependency of superprecipitation upon KCl concentration is shown in Table II. EDTA retarded the superprecipitation.

TABLE II. Effect of KCl concentration on the superprecipitation of pecten and crayfish myosin Bs[15,21]

0.5–1.0 mg/ml 20°C (Crayfish), 26°C (Pecten). 0.5–1.0 mM ATP.
++ intense, + moderate, ± weak, − negative.

KCl concn.	Pecten		Crayfish (pH 7.0)	
	pH 6.5	pH 7.5	without EDTA	with 10 mM EDTA
0.06			+	−
0.08			+	−
0.10			++	−
0.12	±	++	++	+
0.16			+	±
0.18	+	+		
0.20			−	−
0.24	+	−	−	−
0.30	−	−		
0.36	−	−		

(2) Viscosity change

The viscosity and the light scattering of myosin B solution are strikingly reduced in the presence of 0.6 M KCl by adding ATP, which varies either or both of the size and shape of the protein[2,23].

Myosin Bs from higher invertebrate muscles show an anomalously

TABLE III. Viscosity change of myosin B with ATP

Animal	Type of muscle	Temp. (°C)	$Z\eta$ *)	$Z\eta_{ATP}$	ATP **) sensitivity (%)	Ref.
Honeybee	thoracic	16	0.40	0.16	135	14
Pecten	fast adductor	14.5	0.28	0.09	200	12
Pecten	slow adductor	14.5	0.24	0.14	70	12
Crayfish	tail	6	0.40	0.20	100	15
Rabbit	skeletal		0.45	0.25	80	

*) $Z\eta = \ln \frac{\eta_{rel}}{C}$; C = g/L.

**) ATP sensitivity = $\frac{Z\eta - Z\eta_{ATP}}{Z\eta_{ATP}} \times 100$.

high viscosity in 0.6 M KCl, which markedly drops by the addition of ATP. The ATP sensitivity, defined by WEBER[2], is listed in Table III.

The extent of the viscosity drop with ATP addition is little affected by adding divalent cations, but the recovery process depends appre-

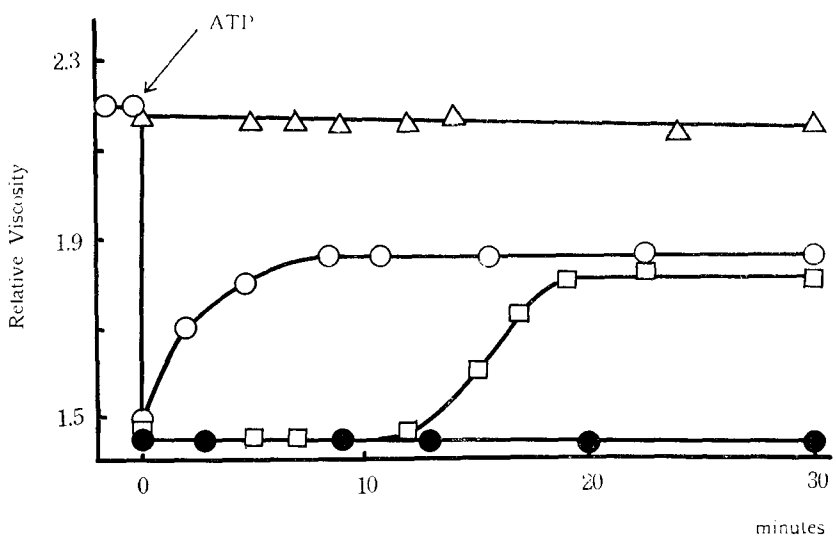

Fig. 3 Change in viscosity of crayfish myosin B with ATP.
Conditions: pH 6.4, 8.5°C, 2.0 mg protein/ml, 1 mM ATP, 0.6 M KCl.
●, 3 mM $MgCl_2$; □, none added; ○, 0.3 mM $CaCl_2$; △, 3 mM EDTA.

ciably upon the sort of these cations. The Ca^{++} considerably accelerates the recovery process, whereas Mg^{++} retards it as indicated in Fig. 3. Such effect may most probably be due to their effects upon the rate of breakdown of ATP. However, the recovery process is more complicated than usually assumed and some protein factor, probably guanidine kinase, may be involved in it, as proved with pecten myosin B[4,12,21].

It should be noted that EDTA at sufficiently high concentration, e.g. 10 mM, completely suppresses the change of the viscosity as well as of the light scattering caused by the addition of 0.1 mM ATP, although this inhibition of EDTA can be removed by increasing the ATP concentration to 1 mM[24].

Inosine triphosphate, the deaminated ATP, can cause the viscosity change of rabbit myosin B, only in the presence of Mg^{++}[25]. This is also the case with insect myosin B[18]. However, it should be noted that a rapid recovery process was found to take place in this case, possibly owing to the augmenting effect of Mg^{++} on the inosinetriphosphatase activity of myosin B.

(3) Functional unit weight

The binding of ATP to myosin B is so stable in the presence of sufficient amount of Mg^{++} that the amount of myosin B combined with one mole of ATP can be calculated easily from the relation between the decrease of scattered light and the ATP concentration. This amount of myosin B was defined as functional unit weight[4]. Since the active site of myosin B, which ATP combines to cause the physical change of the protein solution and itself to hydrolyze, is very probably one and the same[4], the unit measured by the light scattering method may be taken the amount of myosin B per mol of the ATPase active site.

The results obtained by us are all summarized in Table IV. It should be noted that the functional unit weight of myosin Bs from smooth muscles, e.g. swine oesophagus[28] and slow adductor of pecten[21], are considerably larger than that from striated ones. As pointed out by KOMINZ[29], the myosin B preparations from invertebrate smooth muscle such as slow adductor of pecten might be appreciably contaminated with tropomyosin A or the like ones inactive to ATP-responce.

TABLE IV. Functional unit weight of myosin B[4]

Animal	Type of muscle	Unit weight (10^5 g)	ATPase activity		k_2*) (sec^{-1})	Ref.
			K_m (10^{-3} M L^{-1})	V_{max} (10^{-6} M sec^{-1} g^{-1})		
Rabbit	skeletal	5.0	0.15	6	3.1	26, 26a
Swine	heart	1.8				27
Swine	oesophagus	10	0.53	3.9	3.9	28
Pecten	fast adductor	3.9	0.41	9.6	3.7	12
Pecten	slow adductor	12.2	0.45	3.2	3.9	12
Honeybee	thoracic	4.0	0.30	5.9	2.4	14

*) Functional unit weight $\times V_{max}$.

4. Adenosinetriphosphatase

Our attention has been focussed during the last five years primarily on the ATPase activity of myosin Bs from various animal muscles. Main conclusions arrived at on this problem are as follows:

i) All the myosin Bs from higher invertebrates show the Ca^{++}-activated ATPase.

ii) In high KCl solution Mg^{++} inhibits competitively the activating effect of Ca^{++}.

iii) The other enzymic properties except the above two are not species-specific but appears to be at least group-specific.

iv) The ATPase activities (per functional unit weight) of myosin Bs from various muscles are not so different.

(1) Extent of hydrolysis of ATP

Myosin Bs from all the invertebrates investigated, except sea-anemone, catalyzed the hydrolysis of the only terminal phosphate bond of ATP, as well-established in vertebrate myosin B. The muscles investigated are as follows: body-wall muscle of the annelid *Urechis unicinctus*[30]; tube-feet of the echinodermates (*Asterias amurensis*)[20]; adductors of several species of molluscs[12,13,21]; tail muscle of the crustacean (*Cambarus clarkii*)[15]; thoracic muscles of many insects[14,31] and larva and pupa of some insects[31,32]. The reaction products of the ATPase action were identified by ion-exchange chromatography in the case of myosin Bs from *Pecten* (Mollusca)[21] and *Apis* (Arthropoda)[18] and the enzymatic conversion of ATP to ADP and P was established.

The sea-anemone contractile protein[33,34] converted ATP to AMP and 2P, and dephosphorylated inosine-triphosphate to the monophosphate level as well. It follows that the sea-anemone protein must be an apyrase.

(2) Effects of modifiers

As mentioned above, 5–10 mM Ca^{++} strongly activated the ATPase of myosin Bs from all the animals investigated and the Ca^{++}-activated ATPase was strongly inhibited by Mg^{++} in high KCl solution except for the sea-anemone apyrase. It was, on the other hand, well established in rabbit myosin B[35] that Mg^{++} enhances the enzymic action in the presence of less than 0.1 M KCl. This was however not the case with myosin Bs from insect thoracic muscle[36], crayfish tail muscle[15] and swine oesophagus muscle[28].

A rather curious property of myosin B-ATPase of the rabbit protein

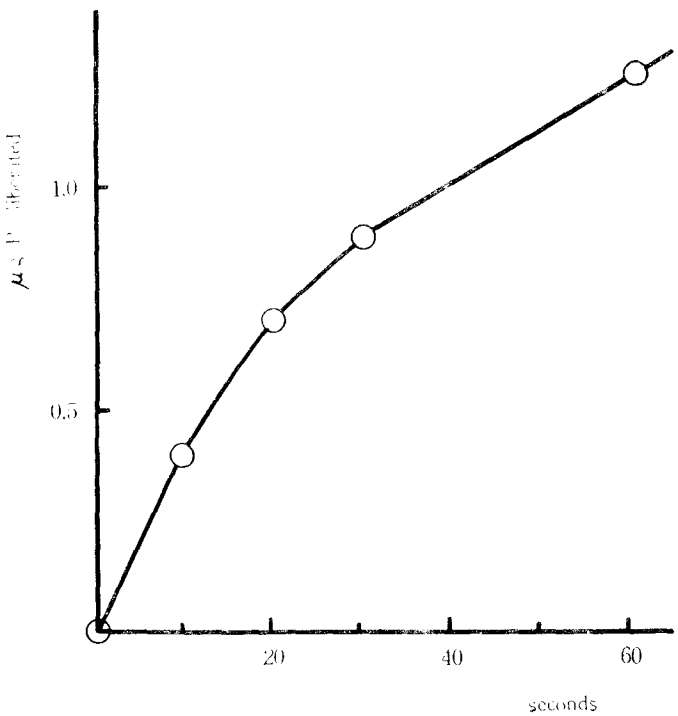

Fig. 4 Initial phase of insect myosin B ATPase.
Conditions: pH 7.0, 37°C, (0.033 M-tris buffer), 0.6 M KCl, 5 mM $MgCl_2$, 1.3 mg. protein per ml, total volume 1.5 ml.

has recently been found with respect to its response to EDTA; EDTA elevated the enzyme action remarkably at high concentration, say 0.6 M, of KCl, pH 7–8.5 and 20–30°C. This effect seemes to be specific to myosin and actomyosin-ATPase, not being observed with the other intracellular ATPases. The enhancing effect of EDTA was clearly observed with myosin Bs from insect[36], molluscan[13] and crustacean[11] muscles. EDTA is the most effective activator, among those under optimum conditions, of the ATPase action of myosin Bs from rabbit and crayfish[15] muscles. But for insect[18,36] and molluscan[13] myosin Bs, EDTA was found to be inferior to Ca^{++} as an activator.

One of the most distinguished properties of myosin B ATPase is that the activity at the initial stage is several times higher than that at the steady state[38]. A detailed study on the initial stage of rabbit myosin B ATPase action has been recently carried out by TONOMURA and KITAGAWA using a special rapid method[39]. This phenomenon was observed also in insect myosin B ATPase. Fig. 4 illustrates an example, with Mg^{++} added. The initial stage depends upon the enzyme concentration and a distinct result seems to be obtained in the presence of powerful activators, such as Ca^{++}, only by the use of the rapid method.

(3) pH optima

Myosin B-ATPase of vertebrate muscle is shown to have two pH optima, a *true* one around pH 6.5 and an *apparent* one around pH 9.5, when Ca^{++} is used as an activator[40]. Usually the activity at the alkaline *apparent* optimum is by far higher than the one at the acidic *true* one.

The presence of these two pH optima was observed in insect, crayfish and clam myosin Bs. In myosin B-ATPase from insect adult and larva, the alkaline peak was lower than the acid one. This character seems not to be order-specific, it being ovserved with ten species belonging to seven orders. Moreover, this is also the case with two species of molluscs (*Cristaria* and *Meretrix*), whose myosin B-ATPase has a similar pH-activity curve to that of rabbit as well as of crayfish myosin B.

Myosin B-ATPase from lower invertebrates, on the other hand, appears to have a different pH-activity curve from the rabbit's one; myosin Bs from the body-wall muscle of an annelid[30] and from a sea-anemone[41] showed, respectively, single pH optimum around pH 7.

(4) Kinetics

The ATAase of myosin Bs, irrespective of their sources, proceeded in good accordance with the Michaelis-Menten theory (Fig. 5):

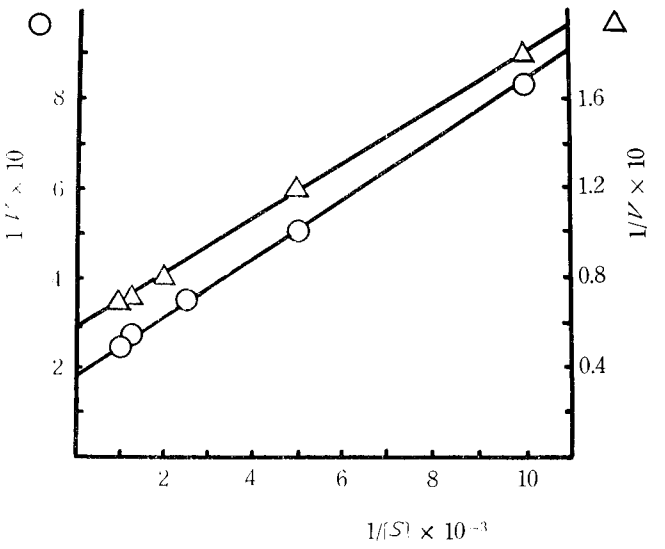

Fig. 5 Effect of substrate concentration on the rate of insect myosin B ATPase.
Conditions: 0.2 M KCl, 10 mM $CaCl_2$; ○, 12°C, pH 6.8 (abicissa, left); △, 32°C, pH 6.0 (abicissa, right).

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]},$$

where v=reaction rate (per g. protein), V_{max} = v at sufficiently high concentration of ATP, $[S]$=concentration of ATP and K_m=the Michaelis constant. The reaction-scheme of the ATPase of myosin B (designated by M) may hence be given as:

$$M + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M.ATP \xrightarrow{k_2} M + ADP + P.$$

Table IV and V summarize the data on the kinetics of myosin B-ATPase. We can see in Table V that the order of magnitude of K_m is not so much different from each other, while the V_{max} values (per g. protein) are somewhat divergent.

TABLE V. Kinetic data of myosin B ATPase

Animal	Type of muscle	K_m 10^{-6} M L^{-1}	V_{max} 10^{-6} M sec^{-1} g^{-1}	Conditions	Ref.
Sea-anemone Winter	whole body	0.14	6.2	pH 7.0, 37°C 6 mM Mg^{++}, 0.2M K^+	22
Sea-anemone Summer	whole body	0.13	52	pH 7.0, 37°C 10 mM Ca^{++}, 0.5M K^+	17
Sea-anemone Summer	whole body	0.25	96	pH 7.0, 37°C 10 mM Mg^{++}, 0.5M K^+	17
Crayfish	tail	0.05	5.5	pH 7.0, 30°C 10 mM Ca^{++}, 0.5M K^+	15
Honeybee	thoracic	0.30	38	pH 6.0, 32°C 10 mM Ca^{++}, 0.2M K^+	14
Honeybee	thoracic	0.20	5.9	pH 7.0, 12°C 10 mM Ca^{++}, 0.2M K^+	14
Worm (Urechis)	body-wall	0.43	3.7	pH 7.2, 37°C 1 mM Ca^{++}, 0.13M K^+	30
Wasp	thoracic	0.11	15	pH 8.5, 37°C 3.3mM Ca^{++}, 0.05M K^+	31
Housefly	thoracic	0.20	26	pH 6.0, 37°C 3.3 mM Ca^{++}, 0.2M K^+	31
Rabbit	skeletal	0.15	6	pH 6.8, 12°C 10 mM Ca^{++}, 0.2M K^+	42

(5) Activity level of ATPase

The levels of Ca^{++}-activated ATPases of various myosin Bs, expressed by k_2 ($=V_{max}\times$ functional unit weight), are not so much different from each other in spite of the great difference of the contraction time of living muscles from which myosin Bs were extracted (see Table IV). Possibly the speed of contraction of living muscle is controlled by some other intricate physiological conditions. However, the ATPase activity (per g. protein) of myosin B of the same species changes in close correlation to the grade of development of muscle fibre, accompanying the different stages of insect metamorphosis[31,32]. Nevertheless the ATPase activity does not directly correspond to the actual muscular function. For example, an adult honeybee just emerged only crawls about and cannot fly out and yet shows as high ATPase activity as an actively flying adult does; the energy-supplying system, very probably the respiratory chain in sarcosomes, is in this case limiting to the

flying[39,43,44].

5. Adenylate Deaminase

It is well-established that myosin and actomyosin in vertebrate muscle implies adenylate deaminase activity[45]. In 1952 W. A. ENGELHARDT[46] emphasized the role of adenylate deaminase activity of myosin to muscle function.

The presence of such a deaminase activity has, however, been questioned in the case of invertebrates. GILMOUR and CALABY[47] were the first to find the absence of deaminase activity in insect actomyosin as well as in insect muscle homogenate. This finding has later been repeatedly confirmed with molluscs, i.e. with myosin B and total homogenate of fast adductor of pecten[48], myosin Bs from retractor muscle of *Mytilus*[49] and from striated adductors of *Meretrix* and *Cristaria*[13]. In other invertebrates, myosin B from crayfish tail muscle showed no deaminase activity[15] and the same was true with the contractile protein

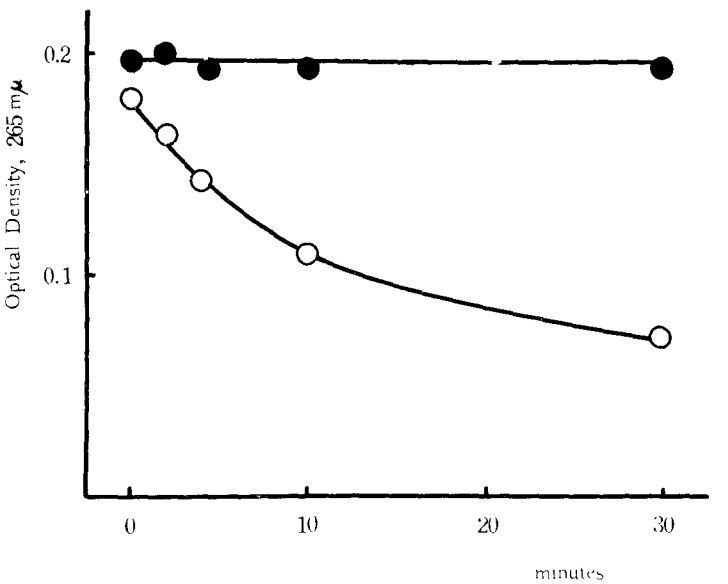

Fig. 6 Adenylate deaminase activity in mouse skeletal myosin B and sea-anemone contractile protein.

Conditions: 0.01 M citrate buffer, 1 mM AMP. Incubated at pH 6.4 at 25°C. Optical density at 265 mμ of an aliquot of the deproteinized filtrate. ●, sea-anemone protein, 1.4 mg; ○, mouse myosin B, 0.5 mg.

from a sea-anemone[34], as demonstrated in Fig. 6. From a view point of biochemical evolution, it will be of great interest to investigate the enzyme action of myosin Bs from cephalochordate and cyclostomate muscles.

On the other hand, adenylate kinase, known as one of the relaxing factors of glycerinated muscle, was found to be contained in the crude myosin B preparations from molluscan (pecten)[12] and arthropod (crayfish[15] and honeybee[18]) muscles.

It is at least evident that the adenylate deaminase activity of myosin does not play an essential role in muscular function, since most invertebrate muscles, although they lack deaminase action, can contract and relax in good efficiency.

6. Conclusion

As described in the preceding sections, there is no fundamental difference among the ATP-myosin B system from muscles of several distinct types of animals belonging to different phyla of classification. This is true especially for myosin Bs from higher invertebrate and vertebrate muscles, and may strongly suggest that the fundamental mechanism at the molecular level for muscular function is essentially common over the animal kingdom. The only difference so far found is the absence of adenylate deaminase activity in invertebrate myosin Bs. Perhaps the physiological speciality of each muscle might be due to a much more delicate regulatory mechanism not detectable by the present physicochemical techniques. In conclusion we whould like to state that the comparative studies on the ATP-myosin B system also support the view generally accepted by biochemists, that the interaction between ATP and actomyosin is the fundamental mechanism of muscle contraction.

Acknowledgements—The authors wish to express their cordial thanks to Prof. J. Horiuti (Hokkaido Univ.) and Prof. J. Ishida (Univ. of Tokyo) for their support and encouragement in carrying out this work; to Prof. T. Kwan (Hokkaido Univ.) for his help in writing this paper in English.

References

1) W. A. ENGELHARDT, Adv. Enzymology **6**, 147 (1946).
2) H. H. WEBER and H. PORTZEHL, Adv. Protein Chem. **7**, 161 (1952); Prog. Biophys. and Biophys. Chem. **4**, 60 (1954).
3) A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction*, 2nd ed. (1951), Academic Press, New York.
4) Y. TONOMURA, This Journal **4**, 87 (1956); **4**, 259 (1957).
5) P. K. SHENG and T. C. TSAO, Sci. Sinica **4**, 157 (1955).
6) T. C TSAO, P. H. TAN and C. M. PENG, Sci. Sinica **5**, 91 (1956).
7) D. R. KOMINZ, F. SAAD and K. LAKI, Nature **179**, 206 (1957).
8) D. R. KOMINZ, F. SAAD, J. A. GLADNER and K. LAKI, Arch. Biochem, Biophys. **70**, 16 (1957).
9) K. BAILEY, Pubbl. Staz. Zool. Napoli **29**, 96 (1956).
10) K. LAKI, J. Cell. Comp. Physiol. in press.
11) E. TARVER and M. F. MORALES, J. Cell. Comp. Physiol. **27**, 235 (1951).
12) Y. TONOMURA, K. YAGI and H. MATSUMIYA, Arch. Biochem. Biophys. **59**, 76 (1955).
13) K. MARUYAMA, Annot. Zool. Jap. **30**, 63 (1957).
14) K. MARUYAMA, J. Cell. Comp. Physiol. in press.
15) K. MARUYAMA, Biol. Bull. in press.
16) O. SNELLMAN and M. Tenow, Biochim. Biophys. Acta **13**, 199 (1954).
17) K. MARUYAMA, Sci. Pap. Coll. Gen. Educ. Univ. Tokyo **7**, 111 (1957).
18) K. MARUYAMA, Sci. Pap. Coll. Gen. Educ. Univ. Tokyo in press.
19) K. MARUYAMA, Z. vergl. Physiol. in press.
20) K. MARUYAMA and H. MATSUMIYA, J. Biochem. (Tokyo) **44**, 537 (1957).
21) Y. TONOMURA, K. YAGI and H. MATSUMIYA, Arch. Biochem. Biophys. **64**, 466 (1956).
22) K. MARUYAMA, Sci. Pap. Coll. Gen. Educ. Univ. Tokyo **6**, 95 (1956).
23) J. J. BLUM and M. F. MORALES, Arch. Biochem. Biophys. **43**, 208 (1953).
24) Y. TONOMURA, H. MATSUMIYA and S. KITAGAWA, Biochim. Biophys. Acta **24**, 568 (1957).
25) S. S. SPICER and W. J. BOWEN, J. Biol. Chem. **188**, 741 (1951).
26) Y. TONOMURA, S. WATANABE and K. YAGI, J. Biochem. (Tokyo) **40**, 27 (1953).
26a) Y. TONOMURA, H. MATSUMIYA, F. MORITA and S. KITAGAWA, Conference on the Chemistry of Muscle Contraction, 1957, Tokyo.
27) A. T. SASAKI, unpublished observation, 1956.
28) Y. TONOMURA and A. T. SASAKI, Enzymol. **18**, 111 (1957).
29) D. R. KOMINZ, personal communication, 1957.
30) K. MARUYAMA, Enzymol. **17**, 90 (1954).
31) K. MARUYAMA, J. Fac. Sci. Tokyo Univ. IV, **7**, 231 (1954).
32) K. MARUYAMA, Biochim. Biophys. Acta, **14**, 284 (1954).
33) K. MARUYAMA, Enzymol. in press.
34) K. MARUYAMA, Enzymol. in press.

35) W. HASSELBACH, Z. Naturforsch. **7 b**, 163 (1952).
36) K. MARUYAMA, Sci. Pap. Coll. Gen. Educ. Univ. Tokyo **6**, 183 (1956).
37) W. J. BOWEN and T. D. KERWIN, J. Biol. Chem. **211**, 237 (1954).
38) A. WEBER and W. HASSELBACH, Biochim. Biophys. Acta **15**, 237 (1954).
39) Y. TONOMURA and S. KITAGAWA, Biochim. Biophys. Acta in press.
40) I. GREEN and W. F. H. M. MOMMAERTS, J. Biol. Chem. **202**, 541 (1953).
41) K. MARUYAMA, Biochim. Biophys. Acta **16**, 589 (1955).
42) S. WATANABE, Y. TONOMURA and H. SHIOKAWA, J. Biochem. (Tokyo) **40**, 387 (1953).
43) K. MARUYAMA und Sh. F. SAKAGAMI, submitted to Z. vergl. Physiol.
44) K. MARUYAMA and K. MORIWAKI, Enzymol. in press.
45) V. S. HERMANN and G. JOSEPOVITS, Hung. Acta Physiol. **2**, 1 (1949).
46) W. A. ENGELHARDT, 2nd International Congr. Biochem. (1952).
47) D. GILMOUR and J. H. CALABY, Enzymol. **16**, 23 (1953).
48) S. KITAGAWA and Y. TONOMURA, J. Biochem. (Tokyo) **44**, 317 (1957).
49) K. ISHIWARA, personal communication, 1956.