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Effect of deuterium oxide on contraction characteristics and ATPase activity in glycerinated single rabbit skeletal muscle fibers

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Abstract

We studied the effect of deuterium oxide (D_2O) on contraction characteristics and ATPase activity of single glycerinated muscle fibers of rabbit psoas. D_2O increased the maximum isometric force P_0 by about 20%, while the force versus stiffness relation did not change appreciably. The maximum shortening velocity under zero load V_{max} did not change appreciably in D_2O, so that the force-velocity (P–V) curve was scaled depending on the value of P_0. The Mg-ATPase activity of the fibers during generation of steady isometric force P_0 was reduced by about 50% in D_2O. Based on the Huxley contraction model, these results can be accounted for in terms of D_2O-induced changes in the rate constants f_1 and g_1 for making and breaking actin–myosin linkages in the isometric condition, in such a way that $f_1/(f_1+g_1)$ increases by about 20%, while (f_1+g_1) remains unchanged. The D_2O effect at the molecular level is discussed in connection with biochemical studies on actomyosin ATPase.

Keywords: Deuterium oxide; Muscle contraction; ATPase activity; Huxley contraction model; Skeletal muscle fiber

1. Introduction

In the ATP hydrolysis step by the cross-bridge ($M \cdot ATP + H_2O \rightarrow M \cdot ADP \cdot Pi$), water attacks γ-phosphorus atom of ATP. To obtain information about the kinetics of myosin-ATPase activity, the effect of substituting deuterium oxide (D_2O) for H_2O has been studied by several authors. Hotta and Morales [1] and Inoue et al. [2] reported that myosin ATPase activity in D_2O decreased to about 60% in D_2O. Chaen et al. [3] showed that ATP-dependent actin–myosin sliding was about 40% slower in D_2O than in H_2O, and that the slow actin–myosin sliding in D_2O was associated with a slow rate of ADP release from actomyosin–ADP complex and the increased affinity of actin for myosin in the presence of ATP. On the other hand, physiological studies on the effect of D_2O on intact skeletal muscle indicate that D_2O affects excitation–contraction coupling by depressing the Ca^{2+} release from the sarcoplasmic reticulum (SR), thus reducing the rate of force development on activation [4–10]. Despite the above studies, however, little information is at present available about the effect of D_2O on contraction characteristics of demembranated muscle fibers, which can only be activated by externally applied Ca^{2+}. The present experiments were undertaken to study the effect of D_2O on the contraction characteristics and the ATPase activity of glycerinated rabbit psoas fibers, and to relate the results obtained with the effect of D_2O on the ATPase kinetics of actomyosin in solution.

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2. Materials and methods

2.1. Muscle fiber preparation and experimental setup

Rabbits were killed by decapitation under pentobarbital anesthesia. Strips of rabbit psoas muscle (diameter, ~2 mm) were dissected and tied to a glass rod and kept in a 50% (vol/vol) glycerol solution containing 50 mM KCl, 4 mM $MgCl_2$. 4 mM EGTA, and 20 mM Tris maleate (pH 7.0) at 0 °C overnight. After a change of the solution, they were stored at −20 °C for 1–3 weeks before use. Single muscle fibers (diameter, 50–100 μm) were carefully dissected from the glycerinated muscle strips and mounted horizontally in an experimental chamber between a force transducer (with a compliance of 0.1 mm/N and a resonant frequency of 5 kHz; Akers, Horten, Norway; AE801) and a servomotor (controlled by JCCX101 control unit; General Scanning, Watertown, MA; G100PD) by gluing both ends to the extension of the transducer and the servomotor with collodion. The servomotor contained a displacement transducer (differential capacitor) sensing the position of the motor arm. The compliance of the motor arm (length, 10 mm) at the point of attachment of the fiber was ~0.2 mm/N when the servomotor system was operated in the length clamp mode. The sarcomere length of the fiber was measured by optical diffraction with HeNe laser light. The fiber was kept at its slack length L_0 (2–3 mm) with sarcomere lengths of 2.2–2.3 μm. Relaxing solution contained 80 mM K-propionate, 20 mM imidazole, 10 mM EGTA, 4 mM ATP, 5 mM $MgCl_2$, 15 mM creatine phosphate, 15 U/ml creatine phosphokinase (pH 7.2). Contracting (pCa, ~4) solution was prepared by adding 4 mM $CaCl_2$ to relaxing solution.

D_2O solutions were prepared by replacing about 99.9% of water in normal experimental solutions with D_2O. In most cases, experiments were first performed in normal H_2O solutions and then in D_2O solutions. In some experiments, this procedure was reversed. Similar results were obtained irrespective of the time (10–60 min) in which the fibers were kept in D_2O solutions. Solutions were kept at 15 °C with a thermoelectric device.

2.2. Stiffness measurement

Muscle fiber stiffness was continuously determined by applying small sinusoidal length changes (1 kHz, peak-to-peak amplitude, ~0.1% of L_0) with the servomotor, and measuring the amplitude of resulting force changes. Sinusoidal voltages were produced with a waveform generator (Wavetek, San Diego; model 164). The sinusoidal component of the force record was separated from muscle fiber force with a high-pass filter with a cut-off frequency of 20 Hz, and its amplitude was measured with an absolute value converter constructed by one of us (T.K.). The force and stiffness changes were simultaneously recorded in a digital wave memory of a digital oscilloscope (Nicolet; model 3091), transferred to a microcomputer (IBM; model PC-AT) for data processing and analysis, and displayed on an X–Y plotter (Hewlett-Packard; model 7470A).

2.3. Determination of force–velocity relation

The servomotor system was operated either in the length control mode or in the force control mode [11,12]. First, the system was in the length control (length clamp) mode so that the fiber contracted isometrically when it was maximally activated with contracting solution. After the fiber developed steady isometric force P_0, the servomotor system was switched to the force control mode, and a ramp decrease in force P (=load) from P_0 to zero was applied by feeding a ramp force decrease signal from the waveform generator to the servomotor system. The resulting shortening of the fiber was recorded in the digital wave memory together with the ramp decrease in force, and force–velocity (P–V) relation was obtained and displayed on the X–Y plotter after data processing with the microcomputer [12,13]. The rate of force decrease was ~5.5 mN/s. The maximum velocity of fiber shortening at P=0 was estimated from the point of intersection of the P–V curve at the velocity axis. Since the force on the fiber was continuously changing with time, shortening velocity was determined by averaging the first-time derivative of the fiber length change for each consecutive time segment (duration 1 ms) during the course of fiber shortening [11,13].

Some experiments were also made in which the velocity of unloaded fiber shortening V_0 was determined by the slack test [14]. During the maximum isometric force generation, the fiber was released quickly by up to 10% of L_0 (in 10 ms), so that the fiber was first slackened and then shortened freely before developing isometric force again. The time from the onset of release to the onset of force redevelopment (Δt) was plotted against the amount of release (ΔL), and the slope of the straight ΔL versus Δt relation was used as a measure of V_0.

2.4. Measurement of ATPase activity

Mg-ATPase activity of the fibers before and during Ca^{2+}-activated isometric force development was recorded by the decrease of NADH during cleavage of ATP [15,16]. The fiber was mounted horizontally between the force transducer and a stainless steel rod in the sample compartment (~0.1 ml) of a dual-wavelength spectrophotometer (model 156; Hitachi) with a sample monochrometer at 340 nm and a reference monochrometer at 400 nm, so that the decrease of NADH was measured from the difference in absorbance between 340 and 400 nm. Both relaxing and contracting solutions contained 0.25 mM NADH, 1.25 mM phosphoenolpyruvate, pyruvate kinase (50 units/ml), lactic dehydrogenase (50 units/ml), 10 mM NaN_3, 50 μM quercetin, oligomycin (1 μg/ml). The light path length through the sample compartment was 10 mm, and solutions in the

compartment were constantly stirred with a magnetic stirrer. The outputs of the spectrophotometer and the force transducer were fed to the digital oscilloscope and displayed on the X–Y plotter.

3. Results

3.1. Effects of D_2O on Ca^{2+}-activated isometric force and muscle fiber stiffness

Single muscle fibers were maximally activated with contracting solution (pCa, ~4) in both H_2O and D_2O. When the fibers were activated in D_2O, they were previously kept in D_2O relaxing solution for 10–40 min before application of D_2O contracting solution. After each application of contracting solution, the fibers were made to relax completely in relaxing solution.

As reported by Allen et al. [10], the maximum isometric force P_0 increased by about 20% (range, 15–25%) in D_2O (Fig. 1A). The rate of isometric force development in response to contracting solution was slowed markedly in D_2O. The half-rise time of force development was 1.6±0.1 s in H_2O and 7.3±0.5 s in D_2O (mean±S.D., n=12). If, however, the fiber was released quickly by ~1% of L_0 (in 1 ms) to reduce the isometric force P_0 to zero, the early time course of force redevelopment did not differ between H_2O and D_2O (Fig. 2), indicating that the slow force development in response to contracture solution may be associated with the D_2O-induced slowing of the regulatory processes but not with the D_2O-induced slowing of cross-bridge kinetics.

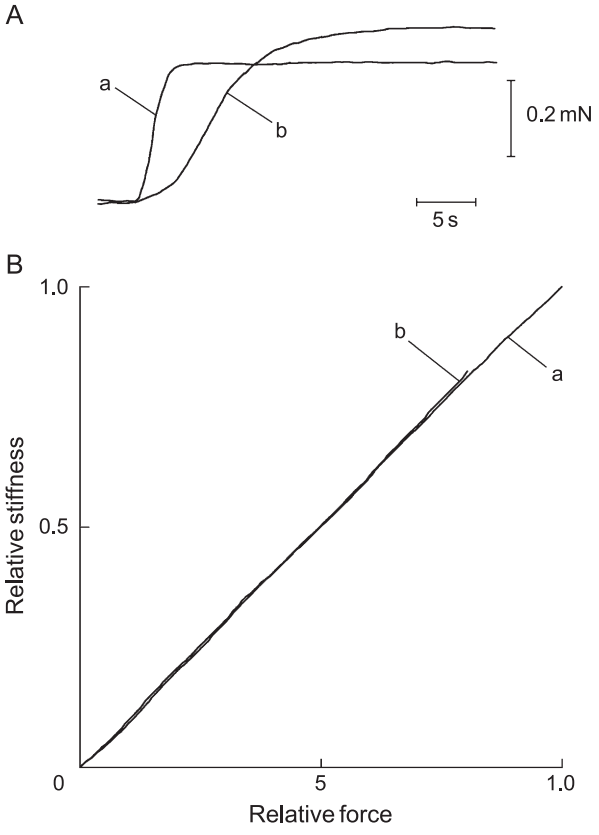

Fig. 1. Effect of D_2O on the Ca^{2+}-activated isometric force and muscle fiber stiffness in a single rabbit psoas fiber. (A) Superimposed records of Ca^{2+}-activated isometric force development in H_2O (a) and in D_2O (b). (B) Isometric force versus stiffness relation in H_2O (a) and in D_2O (b). Both force and stiffness are expressed relative to their maximum values.

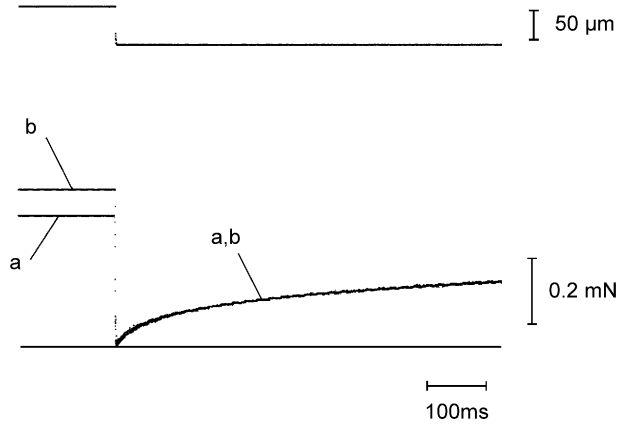

Fig. 2. Superimposed records of isometric force redevelopment following a quick release (~1% of L_0) applied during generation of steady isometric force P_0 in H_2O (a) and in D_2O (b). Note that the time course of the early force redevelopment following the release is the same in H_2O and D_2O.

On the other hand, the force versus stiffness relation was almost linear [13], and did not change appreciably in D_2O (Fig. 1B), so that the D_2O-induced increase in P_0 was associated with the parallel increase in muscle fiber stiffness.

3.2. Effect of D_2O on the P–V relation during steady isometric force generation

The P–V relations in maximally Ca^{2+}-activated fibers were determined by applying ramp decreases in force from steady isometric force to zero. Typical fiber shortening in response to an applied ramp force decrease is shown in the inset of Fig. 3. The P–V curves obtained were double hyperbolic in shape as with the P–V curves of intact single frog muscle fibers (Fig. 3) [11,14]. The maximum shortening velocity V_{max} was 4.6±0.2 L_0/s (mean±S.D., n=10) and did not change appreciably in D_2O despite the increase in the maximum isometric force P_0. The P–V curves in H_2O and D_2O were found to be identical in shape when velocities were replotted against forces P expressed relative to P_0, indicating that the P–V curves were scaled in proportion to the value of P_0. Similar results were obtained with 7 other fibers examined.

Fig. 4 summarizes the measurement of V_0 by the slack test. In both H_2O and D_2O, all data points fell around the same straight regression line, indicating no appreciable effect of D_2O on the value of V_0 (~4.5 L_0/s) which was within the range of V_{max} determined from the P–V relation.

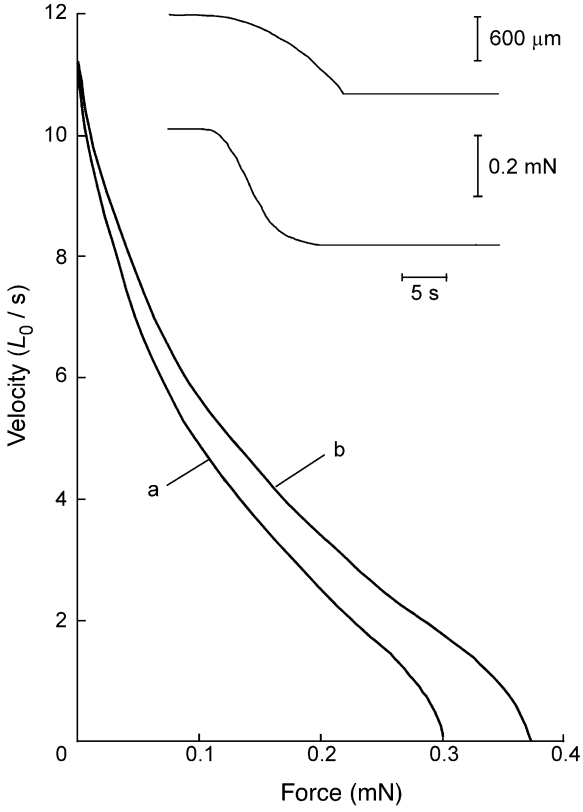

Fig. 3. Effect of D_2O on the force–velocity (P–V) relation. P–V curves were obtained in H_2O (a) and in D_2O (b), respectively. Inset shows the length (upper trace) and force (lower trace) changes in single fiber in D_2O in response to a ramp decrease in force, applied during generation of steady isometric force P_0.

3.3. Effect of D_2O on ATPase activity

The Mg-ATPase activity of maximally Ca^{2+}-activated fibers was measured in both H_2O and D_2O by recording the change in NADH fluorescence. The NADH fluorescence measurement started when the steady Ca^{2+}-activated isometric force P_0 was reached. As shown in Fig. 5, the Mg-ATPase activity was nearly two times higher in H_2O (260±25 μMs^{-1}, mean±S.E., n=4) than in D_2O (140±30 μMs^{-1}, mean±S.E., n=4).

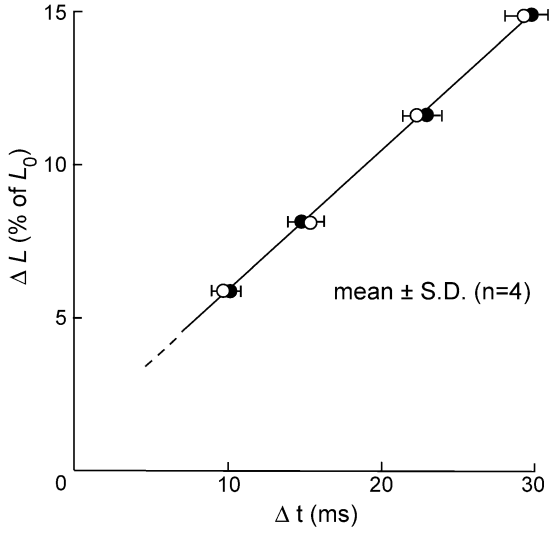

Fig. 4. Relation between ΔL versus Δt obtained from the slack test. Open and filled circles show data points obtained in H_2O and in D_2O, respectively.

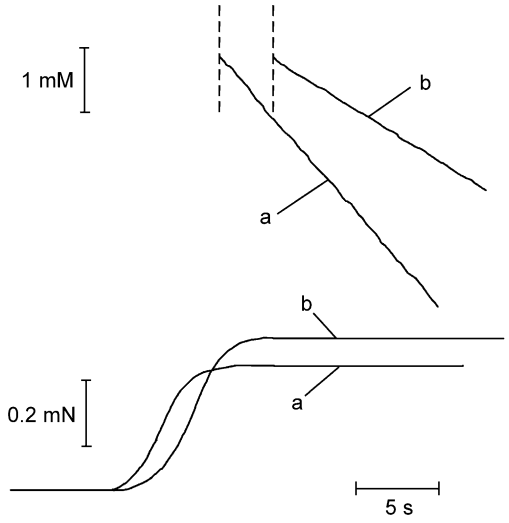

Fig. 5. Effect of D_2O on the Mg-ATPase activity of a single muscle fiber during generation of steady isometric force P_0. Upper traces show decrease of NADH fluorescence, while lower traces show isometric force. Recordings were made in H_2O (traces a) and in D_2O (traces b).

4. Discussion

4.1. Effect of D_2O on contraction characteristics and ATPase activity

In the present experiments, we have shown that, in D_2O, (1) the value of P_0 increased by about 20%, though the rate of force development was markedly reduced (Fig. 1A), (2) the force versus stiffness relation did not change appreciably (Fig. 1B), (3) the value of V_{max} (=V_0) did not change appreciably, so that the P–V curves were scaled depending on the value of P_0 (Figs. 3 and 4), and (4) the Mg-ATPase activity during generation of steady isometric force P_0 was reduced by about 50% (Fig. 5). These results will hereafter be accounted for on the basis of the contraction model of Huxley [17], in which muscle contraction is caused by alternate formation and breaking of cross-linkages between the cross-bridges on the thick filaments and the sites on the thin filaments (actin–myosin linkages).

Huxley [17] succeeded in relating the rate constants of his contraction model with the force–velocity and the force energy relation of Hill [18]. In terms of the Huxley contraction model, the maximum isometric force P_0 is determined by the proportion of attached cross-bridges, expressed as $f_1/(f_1+g_1)$, where f_1 and g_1 are rate constants for making and braking actin–myosin linkages in the isometric condition, respectively. As muscle fiber stiffness

is a measure of the number of attached cross-bridges, the parallel increase of muscle fiber stiffness and force in D_2O (Fig. 1A) indicates an increase in the proportion of attached cross-bridges by about 20%. This means that the value of $f_1/(f_1+g_1)$ also increases by about 20% in D_2O.

Meanwhile, the maximum velocity of shortening under zero load V_{max} is determined by the value of (f_1+g_1), provided that another rate constant for breaking cross-linkages related to muscle fiber shortening g_2 is about 21 times larger than g_1 [17]. As D_2O had no appreciable effect on V_{max} (Figs. 3 and 4), it follows that the value of both (f_1+g_1) and g_2/g_1 (~21) do not change in D_2O. This idea is consistent with the present result that D_2O had no effect on the early rate of isometric force redevelopment after quick release (Fig. 2), which is also thought to be determined by (f_1+g_1) [19]. Meanwhile, the total rate of energy liberation during generation of steady isometric force P_0 is determined by the value $f_1\ g_1/(f_1+g_1)$. Since the Mg-ATPase activity during isometric force generation is reduced by about 50% in D_2O (Fig. 5), the value of $f_1\ g_1$ should be reduced by about 50% in D_2O, as the value of (f_1+g_1) remains unchanged.

4.2. Explanation of present results in terms of Huxley contraction model

Based on the Huxley [17] contraction model, it is possible to determine relative values of f_1 and g_1 in H_2O and D_2O to explain the present results. First, we assume that, in H_2O, the proportion of attached cross-bridges during generation of steady isometric force P_0 is 0.75, a value nearly the same as that in the Huxley contraction model (about 0.8). This means that the value of $f_1/(f_1+g_1)$ is 0.75 [0.75/(0.75+0.25)]. In D_2O, this value increases by 20%, i.e. from 0.75 to 0.9, while the value of (f_1+g_1) remains unchanged. It follows from this that, in D_2O, f_1 increases by 20% (from 0.75 to 0.9) while g_1 decreases by 60% (from 0.25 to 0.1), thus giving, the value of $f_1/(f_1+g_1)$ of 0.9 [0.9/(0.9+0.1)]. As D_2O had no effect on V_{max}, it follows that g_2 also decreases in D_2O by 60% to keep the value of g_2/g_1 unchanged. The above assumption on the effect of D_2O on f_1, g_1 and g_2 also accounts for the present result that D_2O decreases Mg-ATPase activity of the fibers during generation of steady isometric force P_0 by about 50%, because the value of f_1g_1 is 1.9 (0.75×0.25) in H_2O and 0.9 (0.9×0.1) in D_2O.

4.3. Relation of present results to biochemical studies on actomyosin and in vitro motility assay studies

Compared to H_2O, D_2O has higher viscosity, melting point, heat capacity and temperature of maximum density [20]. Crystallographic studies [21] have shown that MgADP is held in the nucleotide binding pocket of myosin head by hydrogen bonds. The above properties of D_2O indicate that the degree of hydrogen bonding in the nucleotide binding pocket is higher in D_2O than in H_2O. When H_2O in the nucleotide pocket is replaced by D_2O [22], the degree of hydrogen bonding to hold ADP would rise to result in a slower rate of ADP release from actomyosin-ADP complex, as has actually been shown by Chaen et al. [3]. The decreased rate of detachment of ADP in D_2O would result in the D_2O-induced decrease of g_1 and g_2 in muscle fibers, assumed in the present study.

Chaen et al. [3] also studied the effect of D_2O on the actin-activated ATPase activity of myosin subfragment 1, and showed that its K_m was 50 μM in H_2O and 33 μM in D_2O, while its V_{max} was the same in both H_2O and D_2O. The increase in the affinity of actin to myosin may be related to the D_2O-induced increase of f_1 in the fibers during steady isometric force generation P_0.

On the other hand, using an in vitro motility assay system, in which actin filaments are made to slide on heavy meromyosin (HMM) in the presence of ATP, Chaen et al. [3] also showed that the actin filament sliding velocity on HMM was about 40% smaller in D_2O than in H_2O. If the actin filament sliding velocity is regarded to represent V_{max} in muscle fibers under zero load, their result contradicts with our result that D_2O had no appreciable effect on V_{max} of muscle fibers (Figs. 3 and 4).

It has been pointed out, however, that the actin filament sliding velocity is not necessarily a good analogue of V_{max} in muscle fibers [23,24]. The in vitro actin filament sliding is caused by a small number of myosin heads randomly oriented on a glass surface, and the mechanism of how the leading edge of an actin filament chooses appropriate myosin heads in its zigzag motion is obscure. During the linear myofilament sliding in muscle fibers, on the other hand, the cross-bridges first attach to actin in the region where they exert positive forces (positive force region), and then more into the region where they exert negative force (negative force region) to be detached rapidly from actin with a rate constant $g_2 \gg g_1$. [17]. The discrepancy on the effect of D_2O on V_{max} and the actin filament sliding velocity therefore seems to arise from differences in the properties of actin–myosin sliding. It should be noted that the present result that V_{max} remains unchanged in both H_2O and D_2O implies that the value of g_2 may not change markedly in D_2O.

In summary, the present results can be accounted for in terms of the Huxley contraction model [17] by assuming that D_2O increases f_1 and decreases g_1 and g_2 without changing the values of (f_1+g_1) and g_2/g_1. The present results also imply that D_2O may not markedly change g_2.

References

[1] K. Hotta, M.F. Morales, Myosin B nucleotide triphosphate in deuterium oxide, J. Biol. Chem. 235 (1960) PC61–PC63.
[2] A. Inoue, Y. Fukushima, Y. Tonomura, Effect of deuterium oxide on elementary steps in the ATPase reactions, J. Biochem. 78 (1975) 1114–1121.
[3] S. Chaen, N. Yamamoto, I. Shirakawa, H. Sugi, Effect of deuterium oxide on actomyosin motility in vitro, Biochim. Biophys. Acta 1506 (2001) 218–223.

[4] M.C. Goodall, Kinetics of muscular contraction in heavy water, Nature 182 (1958) 677.
[5] O. Svensmark, The effect of deuterium oxide on the mechanical properties of muscle, Acta Physiol. Scand. 53 (1961) 75–84.
[6] B. Kaminar, J. Kimura, Deuterium oxide: inhibition of calcium release in muscle, Science 176 (1972) 406–407.
[7] A.B. Eastwood, H. Grundfest, P.W. Brandt, J.P. Reuben, Sites of action of D_2O in intact and skinned crayfish muscle fibers, J. Membr. Biol. 24 (1975) 249–263.
[8] A. Sandow, M.K.D. Pagala, E.C. Sphicas, Deuterium oxide effects on excitation–contraction coupling of skeletal muscle, Biochim. Biophys. Acta 440 (1976) 733–743.
[9] G. Cecchi, F. Colomo, V. Lonbardi, Force–velocity relation in deuterium oxide-treated frog single muscle fibres during the rise of tension in an isometric tetanus, J. Physiol. 317 (1981) 207–221.
[10] D.G. Allen, J.R. Blinks, R.E. Godt, Influence of deuterium oxide on calcium transient and myofibrillar responses of frog skeletal muscle, J. Physiol. 422 (1984) 225–251.
[11] H. Iwamoto, R. Sugaya, H. Sugi, Force–velocity relation of frog skeletal muscle fibres shortening under continuously changing load, J. Physiol. 422 (1990) 185–202.
[12] T. Yamada, O. Abe, T. Kobayashi, H. Sugi, Myofilament sliding per ATP molecule in rabbit muscle fibres studied using laser flash photolysis of caged ATP, J. Physiol. 466 (1993) 229–243.
[13] H. Sugi, T. Kobayashi, T. Gross, K. Noguchi, T. Karr, W.F. Harrington, Contraction characteristics and ATPase activity of skeletal muscle fibers in the presence of antibody to myosin subfragment-2, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 6134–6137.
[14] K.A.P. Edman, The velocity of unloaded shortening and its relation to sarcomere length and isometric force in vertebrate muscle fibres, J. Physiol. 291 (1979) 143–159.
[15] S. Chaen, M. Shimada, H. Sugi, Evidence for cooperative interactions of myosin heads with thin filament in the force generation of vertebrate skeletal muscle fibers, J. Biol. Chem. 261 (1986) 13632–13636.
[16] C. Reggiani, E.J. Potma, R. Bottinelli, M. Canepari, M.A. Pellegrino, G.J.M. Stienen, Chemo-mechanical energy transduction in relation to myosin isoform composition in skeletal muscle fibers of the rat, J. Physiol. 502 (1997) 449–460.
[17] A.F. Huxley, Muscle structure and theories and contraction, Prog. Biophys. Biophys. Chem. 7 (1957) 255–318.
[18] A.V. Hill, The heat of shortening and the dynamic constants of muscle, Proc. R. Soc. B126 (1938) 136–195.
[19] B. Brenner, Muscle mechanics: II. Skinned muscle fibers, in: H. Sugi (Ed.), Current Methods in Muscle Physiology, Oxford University Press, Oxford, 1998, pp. 33–69.
[20] J.J. Katz, Chemical and biological studies with deuterium, Am. Sci. 48 (1960) 544–580.
[21] A.J. Fisher, C.A. Smith, J.B. Thoden, S. Smith, K. Sutoh, H.M. Holden, I. Rayment, X-ray structures of the myosin motor domain of *Dictyostelium discoideum* complexed with MgADP-BeFx and MgADP-AlF4-, Biochemistry 34 (1995) 8960–8972.
[22] T. Yamada, H. Shimizu, M. Nakanishi, M. Tsuboi, Environment of the tryptophan residues in a myosin head: a hydrogen–deuterium exchange study, Biochemistry 20 (1981) 1162–1168.
[23] E. Homsher, F. Wang, J.R. Sellers, Factors affecting movement of F-actin filaments propelled by skeletal muscle heavy meromyosin, Am. J. Physiol. 31 (1992) C714–C723.
[24] H. Sugi, In vitro motility assay, in: H. Sugi (Ed.), Current Methods in Muscle Physiology, Oxford University Press, Oxford, 1998, pp. 173–195.