Adiabatic Compressibility of Myosin Subfragment-1 and Heavy Meromyosin with or without Nucleotide

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ABSTRACT The partial specific adiabatic compressibilities of myosin subfragment-1 (S1) and heavy meromyosin (HMM) of skeletal muscle in solution were determined by measuring the density and the sound velocity of the solution. The partial specific volumes of S1 and HMM were 0.713 and 0.711 cm^3/g, respectively. The partial specific adiabatic compressibilities of S1 and HMM were 4.2×10^{-12} and 2.9×10^{-12} cm^2/dyn, respectively. These values are in the same range as the most of globular proteins so far studied. The result indicates that the flexibility of S1 region almost equals to that of HMM. After binding to ADP·orthovanadate, S1 and HMM became softer than their complexes with ADP. The bulk moduli of S1 and HMM were of the order of $(4–6) \times 10^{10}$ dyn/cm^2, which are very comparable with the bulk modulus of muscle fiber.

INTRODUCTION

It is well known that the essential molecular processes in generation of tensile force for muscle contraction results from the interaction between the thick (myosin-containing) filaments and the thin (actin-containing) filaments. The mutual sliding between two filaments is caused by a cyclic interaction of the cross-bridges extending from the thick filaments to the thin filaments. The cross-bridge is fueled by ATP hydrolysis upon its surface (Huxley, H. E., 1969; Huxley, A. F., 1974) and consists of the globular part of myosin where both the active site for ATPase and actin binding site reside. With the isolated cross-bridge (myosin) in solution, several conformational states have been revealed in the course of ATP hydrolysis (Trentham et al., 1976; Morita, 1977; Eisenberg and Greene, 1980; Eisenberg and Hill, 1985). Among the conformational states, there are four molecular states depending on the chemical species of bound nucleotide, i.e., ATP, ADP·P_i, ADP, and no nucleotide. There is a body of evidence for the structural change of myosin in the solution depending on the bound nucleotide (Goodno and Taylor, 1982; Craig et al., 1985; Applegate and Flicker, 1987; Huston et al., 1988; Cooke, 1989; Katayama, 1989; Aguirre et al., 1989; Highsmith and Eden, 1990).

The adiabatic compressibility is defined as the ratio of the compression of solute volumes in the presence of hydrostatic pressure and its absence. In aqueous solution of protein, water molecules bind to the surface of the protein and are compressed (hydration). Therefore, the adiabatic compressibility is composed of two components; the compactness of protein itself and the hydration of protein (Gekko and Noguchi, 1979). The adiabatic compressibility is experimentally determined from the combination of measurements of 1) the partial specific volume of solute and of 2) the concentration dependence of sound velocity in solution (Sarvazyan, 1979; Gekko and Noguchi, 1979). So far the method is applied to thermodynamic studies of several proteins; for examples, the conformational change of myoglobin was traced by the compressibility measurement (Leung et al., 1986) and a large adiabatic compressibility change of cytochrome *c* was found upon conversion from ferri to ferro form (Eden et al., 1982).

In the present work, we studied the adiabatic compressibilities of myosin subfragment-1 (S1) and heavy meromyosin (HMM) to explore conformational difference due to binding of ADP and ADP plus orthovanadate (ADP·V_i). The transition of cross-bridges from the ADP·P_i bound state (weak binding state) to ADP bound state (strong binding state) is essential for tensile force generation in muscle (Eisenberg and Hill, 1985; Brenner, 1987). A large-scale structural difference is expected to exist between the weak and the strong binding states of myosin head (Brenner, 1987). Therefore, it is interesting to compare the adiabatic compressibility of S1 and HMM obtained in the solution with the elasticity of muscle fiber which being determined in mechanical studies (Truong, 1974; Ford et al., 1977; Jung et al., 1988) and in ultrasonic waves (Tamura et al., 1982; Hatta et al., 1988).

MATERIALS AND METHODS

Chemicals and samples

ATP, ADP, and α-chymotrypsin were purchased from Boehringer Mannheim Biochemicals, Oriental Yeast, and Sigma Chemical Co. Ltd, respectively. Bovine serum albumin (BSA) used for a control measurement was purchased from Sigma (lot A4378). All other chemicals were of reagent grade.

Myosin was prepared from rabbit skeletal muscle by the method of Perry (1955) with a slight modification described by Holtzer and Lowey (1959). Myosin was dissolved in a solution (0.5 M KCl, 25 mM potassium phosphate buffer, and 1 mM dithiothreitol (DTT) at pH 7.0) keynoting glycerol to 50% (V/V) and stored at −20°C. Stocked myosin was used within 3 months. S1 and HMM were prepared by α-chymotryptic digestion of myosin as described by Weeds and Taylor (1975). S1 was purified by gel filtration

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Abbreviations used: S1, myosin subfragment-1; HMM, heavy meromyosin; LMM, light meromyosin; DTT, dithiothreitol; V_i, orthovanadate; BSA, bovine serum albumin.

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(Pharmacia ACA34) in 100 mM KCl, 20 mM Tris-HCl, and 5 mM 2-mercaptoethanol at pH 8.0. Purified S1 contained two isoenzymes, S1 (A1) and S1 (A2), containing alkali 1 light chain (A1) and alkali 2 light chain (A2), respectively. HMM was purified on an anion-exchange column (Whatman DE52) equilibrated in 50 mM Tris-HCl and 1 mM DTT (eluted with a 0–0.5, M KCl linear gradient) according to the method described by Margossian and Lowey (1982). S1 and HMM were used within a week after digestion.

Sample proteins were diluted to protein concentrations of 1.5, 3.0, 4.5, and 6.0 mg/ml with a buffer solution (100 mM KCl, 5 mM $MgCl_2$, 0.1 mM $CaCl_2$, 10 mM Tris-HCl (pH 8.0), 3 mM NaN_3, and 1 mM 2-mercaptoethanol) and dialyzed twice against the same buffer solution at 0°C. The total time for dialysis was more than 36 h. The protein concentrations were determined spectrometrically by using $A_{280}^{1\%} = 6.0\ cm^{-1}$ for HMM, $A_{280}^{1\%} = 7.4\ cm^{-1}$ for S1 (Margossian and Lowey, 1978) and $A_{280}^{1\%} = 6.6\ cm^{-1}$ for BSA before the density and the sound velocity measurements. The final concentrations of the sample were calculated from the total amount of solution after ADP and V_i were added. Sample was out-gassed with an aspirator for 10 min, centrifuged at 90 ×g for 3 min to press out small air bubbles trapped in the solution, and introduced carefully into the measurement cell of density and sound velocity. Samples and the solvent containing ADP were prepared by addition of 175 μl of 50 mM ADP into 2 ml of sample and solvent before being out-gassed. Samples and the solvent containing ADP plus orthovanadate (V_i) were prepared by addition of 80 μl of 50 mM ADP and 40 μl of 100 mM V_i into 2 ml of sample and solvent before being out-gassed. The V_i solution was extracted from V_2O_5 according to the method of Goodno (1982).

Density measurements and partial specific volume

Densities of the solvent and the protein solutions were measured with a precision density meter, DMA-02C (Anton Paar, Graz, Austria). The measurement of temperature was carried out with a Pt^{2+}-resistance at the outside of the sample cell and maintained at 18 ± 0.003°C. The sample temperature was controlled to better than ±0.001°C, because the sample was within a glass cell in an air bath. Room temperature was controlled 25 ± 0.5°C to keep the instrumental condition. To eliminate systematic error, reference measurements with distilled water were achieved before and after each measurement of the protein solution. The accuracy of the measurement was ±1 × 10^{-6}. The instrument constant was determined by calibration with NaCl solutions of known density (International Critical Tables).

The partial specific volume of a protein, v_0 (Gekko and Noguchi, 1979), is defined as

$$v_0 = \lim_{c \to 0} (1 - \Gamma) \Big/ c \qquad (1)$$

where

$$\Gamma = (\rho - c)/\rho_0 \qquad (2)$$

Γ, the apparent volume fraction of the solvent in solution; c, the protein concentration in grams per milliliter of solution; ρ, the density of solution and, ρ_0, the density of solvent.

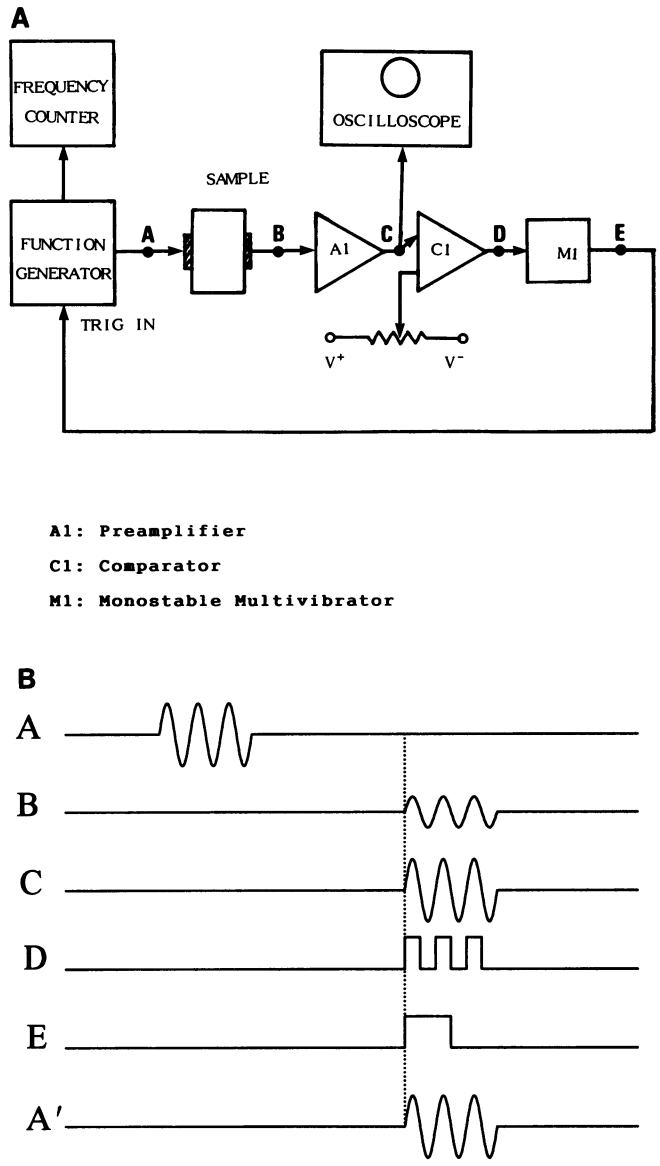

FIGURE 1 (*a*) Experimental arrangement of the parts for recording sound velocity. A1, preamplifier; C1, comparator; M1, monostable multivibrator. (*b*) Diagram illustrating the method for measuring sound velocity. Signals A–E correspond to A–E in the circuit shown in *a*. (*A*) the original wave train and (*B*) the corresponding propagated wave train. The propagated wave train (*B*) is amplified (*C*) and comparated (*D*) to get a rectangular pulse (*E*). The pulse (*E*) is fed to the function generator to transmit original wave (*A'*). Frequency from *A* to *A'* is detected by a counter to determine the sound velocity.

Sound velocity measurements

The velocity of ultrasound in protein solution was measured by a "sing-around pulse method" developed by Greenspan and Tschiegg (1956). A schematic diagram of the measurement system made with integrated circuits is shown in Fig. 1 *a*. The relative time sequence of the pulse trains at various points of the circuitry is also shown in Fig. 1 *b*. Recording of the sound velocity is achieved as follows. Brief trains of sinusoidal waves (*A*) are generated by a function generator (model 193, Waveteck, San Diego, CA) to transmit ultrasonic waves in protein solution through the ceramic transducer. The frequency of ultrasonic waves was 5 MHz and the width of the transmitted wave trains was about 1 μs. Absorption was not observed on the screen of the oscilloscope (model COS5041, Kikusui, Tokyo, Japan) in the protein solutions compared with the solvent. The generated ultrasonic waves travel through the protein solution and are received by the other transducer. The transmitted wave signals (*B*) are fed to a voltage comparator (*C1*) after amplification with a preamplifier (*A1*) with a bandwidth capacity of DC to 40 MHz. The output of the comparator (*D*) is further fed to a monostable multivibrator (*M1*) to get a single rectangular pulse (*E*). The pulse (*E*) triggers the function generator which generates the brief trains of sinusoidal waves (*A'*). The above process from *A* to *A'* is successively repeated after the ultrasonic waves (*A'*) are generated. The frequency of the period from *A* to *A'* (about 8 μs) is obtained by a counter (TR5822, Takeda, Tokyo, Japan) averaging for about 8 s to determine the trip time of ultrasonic waves precisely. The path length for sound was 1.2 cm (outside) of an optical cuvette (inside, 1 cm) being used as a sample cell which needs a sample of

~1 cm^3. Then, the sound velocity is obtained as an amount of the path length divided by the trip time. To eliminate systematic error due to circuit properties, reference measurements with distilled water were achieved before and after each measurement of the protein solution to limit the error of the sound velocity being within ±1 cm/s. Temperature of the measurement cell was recorded by using a Pt^{2+}-resistance with a digital multi-meter (HP-3478A, Hewlett-Packard, Loveland, CO) and kept at 18 ± 0.003°C with a thermobath (RTE-110, Neslab, Newington, NH).

Adiabatic compressibility

The adiabatic compressibility, β, is related to the sound velocity, u, and the density of solution, ρ, by the Laplace equation.

$$\beta = 1/(\rho u^2) \tag{3}$$

The partial specific adiabatic compressibility of the solute, β_s, was calculated with the following equation as a first approximation (Sarvazyan, 1979; Gekko and Hasegawa, 1986)

$$\begin{aligned}\beta_s &= -(1/v_0)(\delta v_0/\delta P)_s \\ &= (\beta_0/v_0)\lim_{c\to 0}(\beta/\beta_0 - \Gamma)/c \\ &\approx (\beta_0/v_0)\lim_{c\to 0}(1 - 2\Delta u/u_0 - \Delta\rho/\rho_0 - \Gamma)/c \\ &= (\beta_0/v_0)\lim_{c\to 0}\{-[2\Delta u/(u_0 c)] - [\Delta\rho/(\rho_0 c)] + v_0\} \\ &= (\beta_0/v_0)\left\{-2\left(\lim_{c\to 0}\Delta u/c\right)\left(\frac{1}{u_0}\right) - \left(\frac{1}{\rho_0}\right) + 2v_0\right\}\end{aligned} \tag{4}$$

v_0, the partial specific volume of solute; P is the pressure; β_0 [$= 1/(\rho_0 u_0^2)$] is the adiabatic compressibility of the solvent; Γ, the apparent volume fraction of the solvent in solution; c, the protein concentration in grams per milliliter of solution; u_0, the sound velocity in solvent; $\Delta u = u - u_0$; ρ_0, the density of solvent; and $\Delta\rho = \rho - \rho_0$. In Figs. 2 and 3, $\Delta\rho$ are plotted against the protein concentration, c. In Figs. 4 and 5, Δu are plotted against the protein concentration, c.

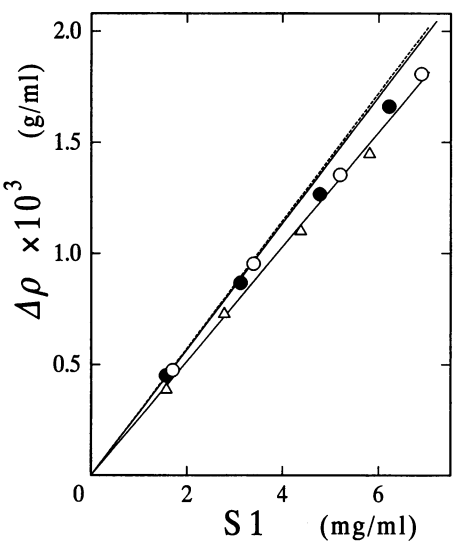

FIGURE 2 Plots of $\Delta\rho$ against the protein concentration, c, of S1. $\Delta\rho = \rho - \rho_0$, where ρ is the density of the protein solution and ρ_0 is the density of the solvent. The symbols are; S1 without nucleotide (*open circles* and *solid line*), S1·ADP (*closed circles* and *dashed line*), and S1·ADP·V_i (*open triangles* and *solid line*). The slopes of lines are obtained from Eqs. 1 and 2.

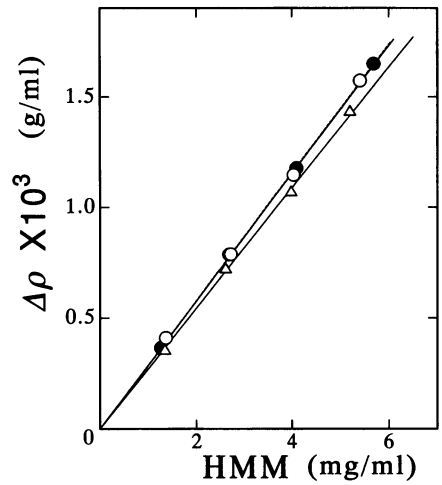

FIGURE 3 Plots of $\Delta\rho$ against the protein concentration, c, of HMM. $\Delta\rho = \rho - \rho_0$, where ρ is the density of the protein solution and ρ_0 is the density of the solvent. The symbols are: HMM without nucleotide (*open circles* and *solid line*), HMM·ADP (*closed circles* and *dashed line*), and HMM·ADP·V_i (*open triangles* and *solid line*). The slopes of lines are obtained from Eqs. 1 and 2.

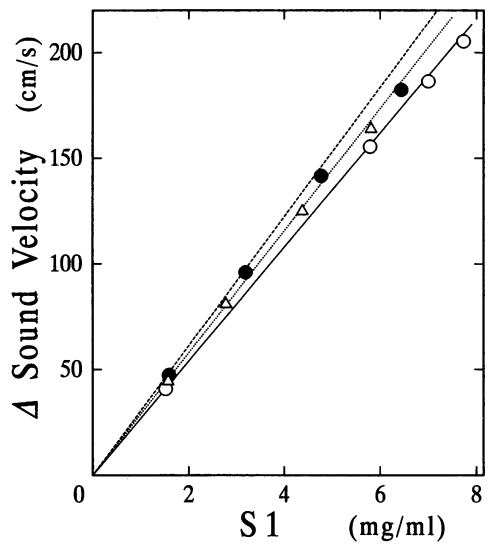

FIGURE 4 Plots of Δu against the protein concentration, c, of S1. $\Delta u = u - u_0$, where u is the sound velocity of the protein solution and u_0 is the sound velocity in the solvent. The symbols are: S1 without nucleotide (*open circles* and *solid line*), S1·ADP (*closed circles* and *dashed line*), and S1·ADP·V_i (*open triangles* and *dotted line*). The slopes of lines are $\lim_{c\to 0}\Delta u/c$ which are determined from the concentration dependence of the sound velocities, $\Delta u/c$, as the extrapolated value to zero protein concentration by means of the least-squares method.

As shown in Eq. 4, the partial specific adiabatic compressibility, β_s, is determined from the concentration dependence of sound velocity, $\lim_{c\to 0}\Delta u/c$, and the partial specific volume of solute, v_0. Therefore, in particular, precise measurements of these two quantity ($\lim_{c\to 0}\Delta u/c$ and v_0) are required to get the partial specific adiabatic compressibility of protein.

RESULTS

The densities of the protein solutions and the solvent without protein yielded an almost linear concentration dependencies

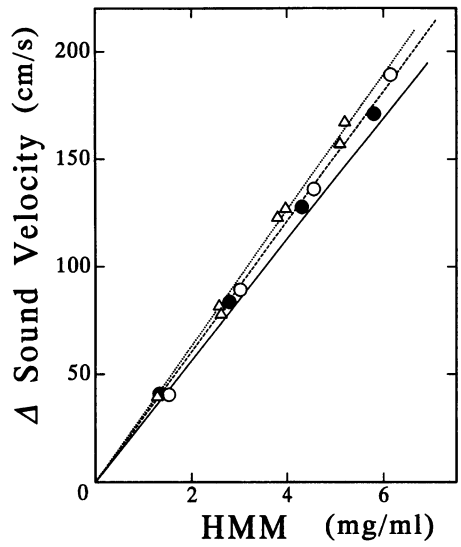

FIGURE 5 Plots of Δu against the protein concentration, c, of HMM. $\Delta u = u - u_0$, where u is the sound velocity in the protein solution and u_0 is the sound velocity in the solvent. The symbols are: HMM without nucleotide (*open circles* and *solid line*), HMM·ADP (*closed circles* and *dashed line*), and HMM·ADP·V_i (*open triangles* and *dotted line*). The slopes of lines are $\lim_{c\to 0} \Delta u/c$ which are determined from the concentration dependence of the sound velocities, $\Delta u/c$, as the extrapolated value to zero protein concentration by means of the least-squares method.

shown in Fig. 2 for S1 and Fig. 3 for HMM. The partial specific volume of a protein, v_0, was determined from Eqs. 1 and 2 as the extrapolated value of the apparent specific volume to zero protein concentration by the least-squares method. The partial specific volumes, v_0, at 18°C are presented in the third column of Table 1 with the results of BSA at 17.5°C. The v_0 values of S1 and HMM were in the range of 0.706–0.739 cm^3/g, in which v_0 of S1·ADP·V_i and HMM·ADP·V_i were noticeably larger than v_0 of others.

TABLE 1 Effect of nucleotide binding on the physical parameters of S1 and HMM

Sample	$\lim_{c\to 0}$ Δu/c (cm/s/mg/ml)	v_0 (cm^3/g)	$\beta_s \times 10^{12}$ (cm^2/dyn)
S1	27.0 ± 0.1 (0.3)	0.713 ± 0.004 (0.006)	4.2 ± 0.6 (1.0)
S1·ADP	30.6 ± 0.6 (0.8)	0.710 ± 0.006 (0.008)	0.7 ± 1.2 (1.6)
S1·ADP·V_i	28.9 ± 0.5 (0.7)	0.739 ± 0.008 (0.010)	5.7 ± 1.3 (1.7)
HMM	28.2 ± 0.3 (0.5)	0.711 ± 0.004 (0.006)	2.9 ± 0.8 (1.2)
HMM·ADP	30.3 ± 0.2 (0.4)	0.706 ± 0.003 (0.005)	0.5 ± 0.4 (0.8)
HMM·ADP·V_i	31.6 ± 0.9 (1.1)	0.725 ± 0.006 (0.008)	1.8 ± 1.4 (1.8)
BSA	25.6 ± 0.3 (0.5)	0.723 ± 0.003 (0.005)	6.6 ± 0.6 (1.0)
BSA (ADP)	25.5 ± 0.2 (0.4)	0.723 ± 0.003 (0.005)	6.5 ± 0.5 (0.9)
BSA (ADP·V_i)	25.8 ± 0.5 (0.7)	0.728 ± 0.003 (0.005)	6.9 ± 0.8 (1.2)
LMM		0.711*	

Partial specific volume (v_0) and partial specific adiabatic compressibility (β_s) of S1 and HMM at 18°C.

* Data of Young et al. (1964) at 5°C in 0.5 M KCl and pH 7.0 in 50 mM phosphate buffer. The error values in a parenthesis are further considered with the uncertainty in the concentration of protein based upon the $A^{1\%}_{280}$.

The sound velocities in the protein solutions and the solvent without protein yielded concentration dependence as shown in Fig. 4 for S1 and Fig. 5 for HMM. The velocity measurements were carried out with the samples prepared independently from the density measurements. The concentration dependence of the sound velocity, $\lim_{c\to 0} \Delta u/c$, was determined from the $\Delta u/c$ values as the extrapolated value to zero protein concentration by the least-squares method. They are shown in the second column of Table 1 with the results of BSA. The $\lim_{c\to 0} \Delta u/c$ values of S1 were in the range of 27.0–30.6 cm/s/mg/ml, while those of HMM were in the range of 28.2–31.6 cm/s/mg/ml.

The values of partial specific adiabatic compressibility, β_s, calculated from Eq. 4 are listed in the fourth column of Table 1 with the results of BSA. We found that the partial specific adiabatic compressibility, β_s, of S1 ranges in (0–6) $\times 10^{-12}$ cm^2/dyn, which is close to the values of several globular proteins (Gekko and Hasegawa, 1986). We also found that HMM with or without nucleotide has the partial specific adiabatic compressibility of (0–3) $\times 10^{-12}$ cm^2/dyn.

DISCUSSION

Partial specific volume and adiabatic compressibility of S1 and HMM

According to the literature (Gekko and Hasegawa, 1986), the partial specific volumes of 25 proteins obtained in water at 25°C are in a range from 0.69 to 0.75 cm^3/g and their partial specific adiabatic compressibility distribute in a range from -2.5×10^{-12} to 10.9×10^{-12} cm^2/dyn. There is a significant correlation between the partial specific adiabatic compressibility and the partial specific volume of these proteins. For examples, myoglobin whose partial specific adiabatic compressibility of 8.98×10^{-12} cm^2/dyn indicates flexible structure has a partial specific volume of 0.747 cm^3/g, while ribonuclease A whose partial specific adiabatic compressibility being 1.12×10^{-12} cm^2/dyn has a smaller partial specific volume of 0.704 cm^3/g.

In this sense, the partial specific adiabatic compressibility of 4.2×10^{-12} cm^2/dyn and the partial specific volume of 0.713 cm^3/g of S1 suggest that S1 has a common structure in atomic packing. On the other hand, HMM has the partial specific adiabatic compressibility of 2.9×10^{-12} cm^2/dyn and the partial specific volume of 0.711 cm^3/g. The partial specific volume of LMM at 5°C in 0.5 M KCl was 0.711 cm^3/g (Young et al., 1964). Though the ionic concentration was different, this value was almost same as our values of S1 and HMM. Young et al. (1964) measured the apparent specific volumes of HMM and LMM. The apparent specific volume of HMM at 5°C in 100 mM phosphate buffer was 0.720 cm^3/g and that of LMM at 5°C in 0.5 M KCl was 0.701 cm^3/g, respectively. Since these apparent specific volumes of HMM and LMM were obtained at high protein concentration (20–35 mg/ml) with density gradient columns, these apparent specific volumes of HMM and LMM were different from the partial specific volumes of them. The partial specific volume of myosin measured with a pyconometer at 15°C in

0.5 M KCl was 0.725 cm^3/g (Kay, 1960) and at 26°C in 0.4 M KCl was 0.728 cm^3/g (Parrish and Mommaerts, 1954). These values are larger than that expected from a sum of the partial specific volumes of HMM and LMM. Gekko (1991) calculated the partial specific adiabatic compressibility of myosin (-18×10^{-12} cm^2/dyn) at 20°C. This value was much smaller than those of S1 and HMM, may reflecting the effect of rod region.

The partial specific volumes obtained experimentally are smaller than the theoretical ones obtained as a sum of molar volumes of the composite amino acid residues (Zamyatnin, 1972) using the known amino acid composition of myosin (Maita et al., 1991); That is, 0.729 cm^3/g for S1, 0.726 for HMM, 0.720 cm^3/g for S2, and 0.719 cm^3/g for LMM. The partial specific adiabatic compressibility of a protein obtained experimentally comes from two contributions, the cavity and the hydration (Gekko and Noguchi, 1979)

$$\beta_s = -(1/v_0)(\delta v_{cav}/\delta P + \delta \Delta v_{sol}/\delta P) \quad (5)$$

$$= \beta_P - (1/v_0)(\delta \Delta_{sol}/\delta P) \quad (6)$$

where v_{cav} is the volume of cavity for the unit mass of the protein and Δv_{sol} is the volume change of water for the unit mass of the protein caused by solvation or hydration. The first term on the right-hand side of Eq. 6 gives the partial specific adiabatic compressibility of the protein itself, which is called as the intrinsic compressibility (β_p). The second term is negative. Since the cavity and hydration effects canceled each other when three-dimensional protein structure was made from amino acid sequence, the theoretical calculation gave us a probable specific volume. The calculated specific volumes may indicate that the cavities are less in S1 and HMM molecules than expected due to close atomic packing and/or the amount of hydration is larger than expected.

Intrinsic compressibility of S1 and HMM

In order to calculate β_p from Eq. 6, we assumed that the amount of hydrated water of S1 and HMM are in a range between 0.30 and 0.50 g/g of the protein (Kuntz and Kauzmann, 1974; Teller, 1976; Gekko and Noguchi, 1979). The hydration term of Eq. 6, $(1/v_0)$ $(\delta \Delta v_{sol}/\delta P)$, could be calculated by using the amount of hydrated water and the compressibility of bulk water, 45×10^{-12} cm^2/dyn, assuming that the volume change of water due to hydration is −1 ml (= 17 − 18 ml) per mole of water (Gekko and Noguchi, 1979) and that the compressibility of hydrated water is equal to that of ice, 18×10^{-12} cm^2/dyn (Shiio et al., 1955). Then, $(1/v_0)(\delta \Delta v_{sol}/\delta P)$ for S1 becomes 11.8×10^{-12} cm^2/dyn $(=(1/0.713)(45 \times 10^{-12} \times 0.30 - 18 \times 10^{-12} \times 0.30 \times 17/18))$ at atmospheric pressure, when the amount of hydration is 0.30 g/g of the protein. The $(1/v_0)(\delta \Delta v_{sol}/\delta P)$ value is 19.6×10^{-12} cm^2/dyn, when the amount of hydration is 0.50 g/g of the protein. Therefore, the intrinsic compressibility, β_p, of S1 is evaluated to be $(16.0–23.8) \times 10^{-12}$ cm^2/dyn. The intrinsic compressibility of HMM calculated in the same way is $(14.7–22.6) \times 10^{-12}$ cm^2/dyn. According to Gekko and Hasegawa (1986), the intrinsic compressibilities of six various proteins are in a range between 10×10^{-12} and 20×10^{-12} cm^2/dyn. The intrinsic compressibility of S1 and HMM are comparable with those of the globular proteins.

Compressibility change induced by binding of nucleotide and orthovanadate

A control measurement was made by using BSA at 17.5°C. The results shown in Table 1 indicate that a noninteracting protein such as BSA does not respond to ADP and vanadate.

The partial specific adiabatic compressibilities of S1 and HMM decreased upon binding of ADP. Since the partial specific volumes of S1 and HMM were almost constant with or without ADP, the decrease of the partial specific adiabatic compressibilities of S1 and HMM depended on the increase in $\lim_{c \to 0} \Delta u/c$. The partial specific adiabatic compressibility increased 5.0×10^{-12} cm^2/dyn in S1·ADP upon binding of V_i and 1.3×10^{-12} cm^2/dyn in HMM·ADP upon binding of V_i. This shows that ADP·V_i bound state (weak binding state) is softer than ADP bound state (strong binding state) in compressibility. The increase in the partial specific adiabatic compressibility of ADP·V_i bound state is mainly because the partial specific volume of ADP·V_i bound state is larger than those of other states. The increase in the partial specific volume is due to decrease of hydration and/or increase in cavity. The length of S1 is constant with or without ADP, but it becomes shorter when ADP·V_i binds to S1 (Craig et al., 1985; Katayama, 1989; Highsmith and Eden, 1990). These change may correspond to the changes of the partial specific volume and the partial specific adiabatic compressibility with or without ADP·V_i.

Comparison with the stiffness of muscle fiber

The bulk modulus is a reciprocal value of the compressibility. The bulk modulus of S1 and HMM in solution are almost a reciprocal value of the intrinsic compressibility β_p, where β_p is the partial specific adiabatic compressibility of protein itself. The bulk modulus of S1 in solution at 18°C was $(4.2–6.3) \times 10^{10}$ dyn/cm^2, when the amount of hydration was 0.30–0.50 g/g of the protein. The bulk modulus of HMM was $(4.4–6.8) \times 10^{10}$ dyn/cm^2. Gekko and Hasegawa (1986) estimated the intrinsic compressibility of globular proteins at 25°C (lysozyme, α-chymotrypsinogen, obalbumin, bovine serum albumin, β-lactoglobulin and α_s-casein) of which values were in the order of $(10–20) \times 10^{-12}$ cm^2/dyn, i.e., the bulk moduli were in the order of $(5–10) \times 10^{10}$ dyn/cm^2. The bulk modulus of lysozyme, bovine serum albumin and immunoglobulin at 25°C were all about 4×10^{10} dyn/cm^2 (Mitaku et al., 1985), though the values of partial specific volume of the protein were not shown in their paper. Thus, the bulk modulus of S1 and HMM was quite comparable with that of these proteins.

The stiffness (bulk modulus) of resting muscle fiber was measured by using ultrasonic waves of 5 MHz at 19–20°C (Hatta et al., 1988). They were 2.480×10^{10} dyn/cm^2 in the longitudinal direction of muscle fiber and was 2.437×10^{10}

dyn/cm^2 in the transverse direction of muscle fiber. Since the volume fraction of the contents within muscle is not known, the bulk modulus of muscle fiber may be calculated as a sum of the bulk moduli of the contents within muscle multiplied by their weight fraction as a first approximation (Jippo et al., 1984). The weight percent of several components in muscle was shown by Dubuisson (1942); about 80% of water, about 10% of the proteins within the myofibrils, and about 10% of enzymes, sarcoplasmic reticulum, mitochondria, nucleus, and the rest. The bulk modulus of water is 2.2×10^{10} dyn/cm^2 as the reciprocal value of the compressibility (45×10^{-12} cm^2/dyn). The bulk modulus of lipids is also 2.2×10^{10} dyn/cm^2 (Jippo et al., 1984). Assuming the bulk modulus of the protein part in myofibrils is equal to the bulk modulus of S1 and HMM ($(4–6) \times 10^{10}$ dyn/cm^2) and the bulk modulus other than the proteins is equal to lipids, we obtain the bulk modulus of muscle fiber $(2.4–2.6) \times 10^{10}$ dyn/cm^2. Though the approximation is rough, it fairly well agrees with the stiffness obtained experimentally in muscle fiber.

During isometric contraction, the stiffness (bulk modulus) of muscle fiber increased 6.5×10^8 dyn/cm^2 in the longitudinal direction of muscle fiber and decreased -6.4×10^8 dyn/cm^2 in the transverse direction at 19–20°C (Hatta et al., 1988). The amount of changes in the stiffness was two orders smaller than that of resting muscle. The amount of increase in the stiffness in the longitudinal direction is the almost same as the amount of increase in Young's modulus measured by other investigators, $(2–7) \times 10^8$ dyn/cm^2, (Truong, 1974; Shoenberg et al., 1974; Ford et al., 1977; Mason, 1978; Hasan and Mason, 1978; Jung et al., 1988). If this change of muscle stiffness during contraction is due to the change of the bulk moduli of contractile proteins, the amount is about 10% of the bulk moduli of contractile proteins.

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