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Environment of the Tryptophan Residues in a Myosin Head: A Hydrogen–Deuterium Exchange Study†

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ABSTRACT: The structural environment of the tryptophan residues in the myosin head is studied by hydrogen–deuterium exchange of the tryptophan residues in heavy meromyosin and subfragment 1. About seven tryptophan residues per myosin head are found to be buried in the hydrophobic domain(s) of myosin head. The addition of ATP causes almost all the tryptophan residues to become more buried, reflecting that the conformational changes during ATP hydrolysis involve almost all the tryptophan residues in myosin head. This is strongly contrasted by the results obtained so far that only about two tryptophan residues per myosin head are involved in the conformational changes of myosin head during ATP hydrolysis [Morita, F. (1967) *J. Biol. Chem. 242*, 4501–4506; Werber, M. M., Szent-Gyorgyi, A. G., & Fasman, G. D. (1972) *Biochemistry 11*, 2872–2883]. The exchange rate of the tryptophan residues increases at the reaction step M·ADP·P_i (e.g., the majar steady-state intermediate at 25 °C) to M·ADP + P_i. Our previous study on the hydrogen–deuterium exchange of the peptide hydrogens in heavy meromyosin [Yamada, T., Shimizu, H., Nakanishi, M., & Tsuboi, M. (1977) *J. Biochem. (Tokyo) 82*, 139–144] showed, however, that the exchange rate of the peptide hydrogens decreases at the reaction step M·ADP·P_i to M·ADP + P_i. This means that at the reaction step M·ADP·P_i to M·ADP + P_i the tryptophan residues become more exposed while the peptides become more buried in myosin head. These results indicate that at the reaction step M·ADP·P_i to M·ADP + P_i the conformational changes take place in the reciprocal way at the tryptophan domains and at the peptide domains in myosin head.

Muscle contraction takes place with the mutual sliding motion of actin and myosin filaments utilizing the chemical energy of ATP hydrolysis. Although the details of the relation between the ATP hydrolysis scheme and the chemomechanical process of the actomyosin system have not been definitely established yet, it is agreed that Scheme I is directly related with the chemomechanical process of muscle contraction (Inoue et al., 1979; Taylor, 1979). In Scheme I, A and M represent actin and myosin head, respectively. At step a, the dissociation of myosin head from an actin filament occurs concomitantly with the binding of ATP (the formation of the M·ATP complex); at b, the bound ATP is split on myosin head forming the M·ADP·P_i complex; at c, the dissociated myosin head is reassociated with the actin filament; at d, the bound ADP and P_i are released from the actin–myosin complex. In step d, motion (probably rotation) of the myosin head is believed to take place on the actin filament (Huxley, 1969; Huxley & Simmons, 1971).

One ATP binding site and one actin binding site are spatially separated in myosin head (Barany & Barany, 1959; Martonosi, 1975; Highsmith, 1976; Greene & Eisenberg, 1978a; Stein et al., 1979). As the M·ATP and M·ADP·P_i complexes have weaker affinity for actin filaments (Eisenberg et al., 1972; Hoffmann & Goody, 1978; Stein et al., 1979) while free M and M·ADP complex have stronger affinity (Highsmith et al., 1976; Greene & Eisenberg, 1978b; Highsmith, 1978; Mar-

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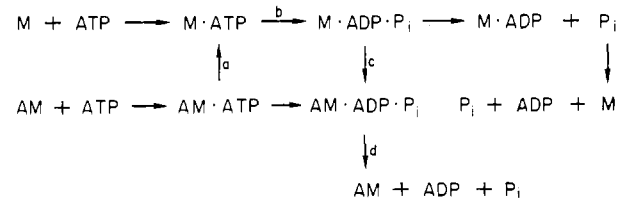
0006-2960/81/0420-1162$01.25/0

Scheme I



gossian & Lowey, 1978), some structural correlation must exist between the conformational changes at the ATP binding site of myosin head and those at the actin binding site. Several studies have successfully showed that conformational changes occur in myosin head during the splitting of ATP (Morita, 1967; Cheung, 1969; Seidel & Gergely, 1971; Werber et al., 1972; Bagshaw et al., 1974; Yamada et al., 1977). At present, these conformational changes are regarded as only localized changes not involving a significant alteration in the helix content (Tonomura et al., 1963; Gratzer & Lowey, 1969; Yamada et al., 1977). Therefore it is plausible to assume that the conformational changes at the ATP binding site and at the actin binding site are localized around these sites. For the study of the molecular mechanism of the chemomechanical energy conversion of muscle contraction, it is essential to establish whether these localized conformational changes during ATP hydrolysis take place correlatively between these two active sites (Morales & Botts, 1979).

In this paper, we report results of our study of hydrogen–deuterium exchange of the tryptophan residues in the myosin head. About seven tryptophan residues per myosin head are found to be buried in the hydrophobic domain(s) of myosin head. On formation of the M·ADP·P_i intermediate complex, the rate of hydrogen–deuterium exchange of almost all of the seven tryptophan residues decreased, which is strongly contrasted by the results obtained so far that only about two tryptophan residues per myosin head are involved in the conformational changes estimated from the ultraviolet difference spectra (Morita, 1967) and from the intrinsic fluorescence (Werber et al., 1972) of the tryptophan residues in myosin head. Thus our results suggest that rather wide domains of myosin head are involved in the conformational changes during ATP hydrolysis.

Moreover, when the results obtained in the present study were compared with those obtained in our previous study of the hydrogen–deuterium exchange of the peptide hydrogens in heavy meromyosin (HMM)[1] (Yamada et al., 1977), it was found that the tryptophan residues become more exposed while the peptides become more buried at the reaction step M·ADP·P_i to M·ADP + P_i. These results indicate that during ATP hydrolysis the conformational changes occur in a reciprocal way at the tryptophan domains and peptide domains in myosin head.

Materials and Methods

Reagents. All chemicals were of reagent grade. ATP, ADP, and AMPPNP were purchased from Boehringer Mannheim Yamanouchi Co. D_2O was purchased from CEA, CEN, Saclay, France. Glass-distilled water was used throughout.

Protein. Myosin was prepared from rabbit back and leg muscles by the method of Perry (1955). HMM(try) was prepared from myosin by tryptic digestion by the method of Lowey & Cohen (1962). HMM(chy) was prepared from myosin by chymotryptic digestion by the method of Weeds & Taylor (1975). Crude HMM preparations were fractionated by precipitation in 45–55% saturated ammonium sulfate. Ammonium sulfate was removed by extensive dialysis against an appropriate buffer solution. In the present work, HMM(try) was used if not otherwise stated. SF-1 was prepared from myosin by chymotryptic digestion by the method of Weeds & Taylor (1975) and chromatographed on a Sephadex G-200 column. ATPase activity was measured in 0.6 M KCl, 20 mM Tris-HCl (pH 7.6), and 1 mM ATP at 20 °C. Either 2 mM $MgCl_2$, 10 mM $CaCl_2$, or 5 mM EDTA was included for the measurement of Mg^{2+}, Ca^{2+}, and EDTA ATPase activity, respectively. The Mg^{2+}, Ca^{2+}, and EDTA ATPase activities of HMM and SF-1 were 0.01, 1.1, and 5.6 mol of P_i s^{-1} mol^{-1} of myosin head, respectively.

Protein concentration was determined spectrophotometrically by employing $E_{280}^{1\%} = 6.47$ (Young et al., 1965) and 7.50 (Weeds & Pope, 1977) for HMM and SF-1, respectively. Molecular weights were assumed to be 340000 (Weeds & Pope, 1977) and 115000 (Weeds & Pope, 1977) for HMM and SF-1, respectively.

Hydrogen–Deuterium Exchange Experiments. Ultraviolet absorption changes due to hydrogen–deuterium exchange of the tryptophan residues in myosin heads were observed as described previously (Nakanishi et al., 1978) by using a Union Giken high-sensitivity spectrophotometer SM-401 and were traced with a Union Giken stopped-flow spectrophotometer RA-401. This was equipped with a rapid-mixing device with a dead time of about 0.5 ms, with a cell of an optical path length of 10 mm, and with an ultraviolet spectrophotometer with a focal length of 25 cm, sensitivity of 0.0004 OD unit, and a response time of about 1.0 ms. This was connected to a Union Giken data processor RA-450, a monitor scope, and an X-Y recorder.

The hydrogen to deuterium exchange reactions were followed at various scanning speeds: 1-, 5-, and 20-s scanning speeds were employed mainly. Data from the fast scans gave the ultraviolet absorption at the initial stage of the hydrogen to deuterium exchange reaction and those from the slow scans that at the final stage. Thus data reconstituted from the fast, intermediate, and slow scans gave the overall time course of the ultraviolet absorption change due to the hydrogen to deuterium exchange reaction. Averaged data from 6–12 scans were obtained for each measurement. All experiments were repeated by using three to five different myosin head preparations. They showed good reproducibility.

The hydrogen and/or deuterium ion concentrations of the solution were measured with a Hitachi-Horiba F-7 pH meter. In this paper, we use the notation "pH" even for the deuterium ion concentration in a deuterium oxide solution, and pH metric readings are always given without any correction.

Results

Hydrogen–Deuterium Exchange of Tryptophan Residues in HMM. When HMM in H_2O solution (initial concentration of 26 μM and final concentration of 13 μM) was rapidly mixed with the same volume of a D_2O solution (in 0.1 M KCl, 2 mM $MgCl_2$, and 20 mM Tris-HCl at pH 8.0 and 25 °C), a time-dependent decrease in absorption at 294 nm was observed as shown in Figure 1. A replot of such data, as illustrated in Figure 2, shows that the absorption decrease is approximately composed of two first-order processes, a fast-exchanging and a slow-exchanging process, the exchanging rates of which differ by about 1 order of magnitude. By extrapolating the straight lines in parts A and B of Figure 2 to zero

[1] Abbreviations used: HMM, heavy meromyosin; SF-1, subfragment 1; ATPase, adenosinetriphosphatase; AMPPNP, adenyl imidodiphosphate; EDTA, ethylenediaminetetraacetic acid; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; $NaDodSO_4$, sodium dodecyl sulfate; D_2O, deuterium oxide.

Table I: Rates of Hydrogen–Deuterium Exchange of the Fast-Exchanging and the Slow-Exchanging Groups of the Tryptophan Residues of HMM at Various Temperatures[a]

	rate of hydrogen–deuterium exchange (s^{-1})							
	fast-exchanging group				slow-exchanging group			
temp (°C)	free	+AMPPNP	+ATP	+ADP	free	+AMPPNP	+ATP	+ADP
29	3.53	2.23	1.73	2.89	0.40	0.38	0.06	0.24
25	2.98	1.93	1.63	2.31	0.31	0.35	0.05	0.23
20	1.87	1.35	1.39	2.10	0.25	0.31	0.04	0.21
15	1.39	1.12		1.82	0.15	0.32	0.03	0.20
10	0.96	0.95		1.00	0.11	0.29		0.19

[a] The conditions are given in the legend to Figure 1.

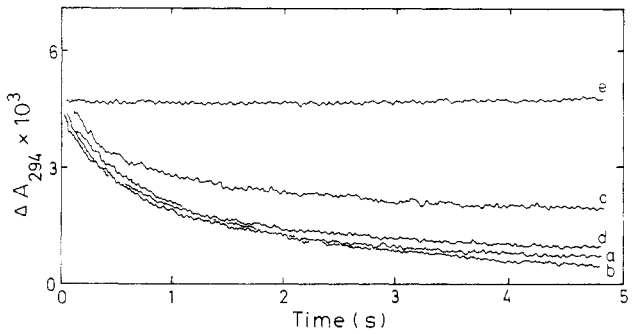


FIGURE 1: Time dependence of the decrease in absorbance at 294 nm observed when (a) HMM in H_2O solution is mixed with D_2O solution, (b) HMM + AMPPNP in H_2O solution is mixed with D_2O solution, (c) HMM in H_2O solution is mixed with ATP in D_2O solution, (d) HMM + ADP in H_2O solution is mixed with D_2O solution, and (e) HMM in 50% D_2O solution is mixed with 50% D_2O solution (mixing 1:1 v/v; final concentration of HMM 13 μM, of AMPPNP, ATP, and ADP 200 μM). Conditions: 0.1 M KCl, 2 mM $MgCl_2$, and 20 mM Tris-HCl at pH 8.0 and 25 °C.

time, we can determine the total absorption change concomitantly with these two processes. The total absorption changes measured in this way at several different wavelengths generate the difference spectrum shown in Figure 3. This difference spectrum has a broad peak at about 292 nm, indicating that the absorption decrease is due to the hydrogen–deuterium exchange reaction of the indole NH group of tryptophan residues in HMM (Nakanishi et al., 1978). By using $\Delta\epsilon$ = 30 for the total absorption change of tryptophan (Nakanishi et al., 1978), we may estimate the number of the tryptophan residues in HMM detectable by the present hydrogen–deuterium exchange technique. The calculated number was 7.1 tryptophan residues per myosin head.

For reduction of the contribution to the ultraviolet absorption change due to the hydrogen–deuterium exchange of tyrosine residues in HMM, which has a broad peak at around 285 nm (Nakanishi & Tsuboi, 1978), the hydrogen–deuterium exchange reaction of the tryptophan residues was examined by measuring the absorption change at 294 nm. Since the whole exchange process is composed of two first-order processes having about the same magnitude of absorption change at zero time as shown in Figure 2, the seven tryptophan residues may be tentatively classified into 3.5 fast-exchanging and 3.5 slow-exchanging groups. The observed time courses of the hydrogen–deuterium exchange were analyzed by least-squares fitting to two exponential curves using a Hitachi computer HITAC 8700/8800. The results of the data fitting were satisfactory for all cases presented in this paper within experimental error. For example, the fast-exchange and slow-exchange groups of the tryptophan residues had exchange rates of 1.87 and 0.25 s^{-1} at 20 °C, respectively. Similar observations and analysis were made at various temperatures, and the obtained rate constants are summarized in Table I.

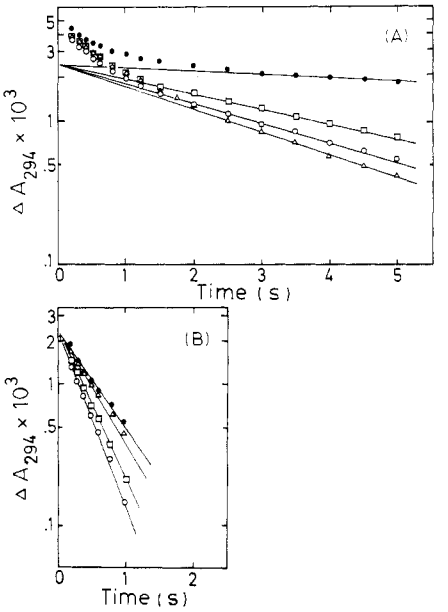


FIGURE 2: (A) Semilogarithmic plots of the same data as those for (a–d) in Figure 1 and (B) the data after subtracting the slow-exchanging processes. (○) Free HMM, (△) HMM + AMPPNP, (●) HMM + ATP, and (□) HMM + ADP.

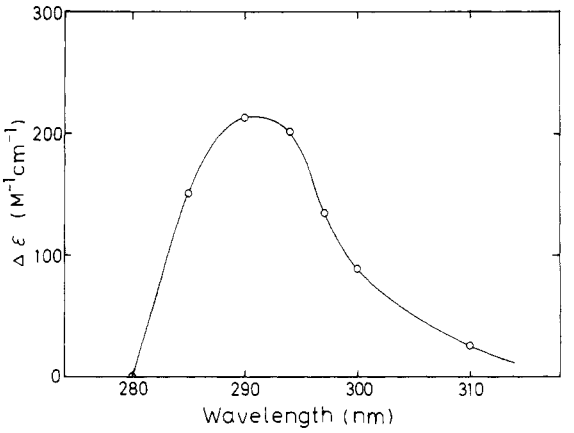


FIGURE 3: Difference spectrum obtained from the kinetic measurements. Each point indicates the total absorbance change obtained for the sum of the two first-order processes observed on mixing HMM in H_2O solution with D_2O solution by extrapolating to zero time.

We analyze the environment of the tryptophan residues in the myosin head from the rate of the hydrogen–deuterium exchange as

$$HMM^j_{buried} \underset{k^j_-}{\overset{k^j_+}{\rightleftharpoons}} HMM^j_{exposed} \xrightarrow{k_e} {}^*HMM^j_{exposed} \rightleftharpoons {}^*HMM^j_{buried} \quad (1)$$

where $HMM^j_{exposed}$ means a form of HMM in which the jth group of tryptophan residue is exposed to the solvent and can be deuterated and HMM^j_{buried} means the one in which the jth

Table II: Values of the Apparent Equilibrium Constants between the Exposed and the Buried Forms around the Fast-Exchanging and the Slow-Exchanging Groups of Tryptophan Residues of HMM[a]

	apparent equilibrium constant between exposed and buried forms of HMM ($\times 10^3$)							
	fast-exchanging group				slow-exchanging group			
temp (°C)	free	+AMPPNP	+ATP	+ADP	free	+AMPPNP	+ATP	+ADP
29	40.8	25.8	20.0	33.4	4.6	4.4	0.7	2.8
25	57.2	37.0	31.3	44.3	6.0	6.7	1.0	4.4
20	59.4	42.9	44.1	66.7	7.9	9.8	1.3	6.7
15	75.1	60.5		98.4	8.1	17.3	1.6	10.8
10	88.9	88.0		92.6	10.2	26.9		17.6

[a] The values were calculated from the rate constants of the hydrogen to deuterium exchange shown in Table I by using eq 2. For details, see text.

group of tryptophan residue is buried in the HMM molecule so that it cannot be deuterated. The asterisk indicates the deuterated HMM molecule. If the rate constants of the conformational change, k^j_+ and k^j_-, are much greater than the rate constant of the exchange in the exposed form, k_e, i.e., k^j_+, $k^j_- \gg k_e$, the exchange rate of the jth group of tryptophan residue can be given as (Hvidt & Nielsen, 1966; Nakanishi et al., 1972)

$$k_j = \frac{k^j_+}{k^j_+ + k^j_-}k_e = \frac{[\mathrm{HMM}^j_{\mathrm{exposed}}]}{[\mathrm{HMM}^j_{\mathrm{exposed}}] + [\mathrm{HMM}^j_{\mathrm{buried}}]}k_e = \frac{[\mathrm{HMM}^j_{\mathrm{exposed}}]}{[\mathrm{HMM}^j_{\mathrm{buried}}]}k_e = K^j_{\mathrm{app}}k_e \quad (2)$$

where K^j_{app} is the apparent equilibrium constant of the exposed and buried forms of the jth group of tryptophan.

k_e is the rate of the hydrogen–deuterium exchange of a tryptophan indole NH when it is completely exposed to the solvent. The values of k_e were determined by measuring the rates of the hydrogen–deuterium exchange for a tryptophan solution with the present salt conditions at various temperatures. It was found to be 31.5 s^{-1} at 20 °C with an activation energy of 18 kcal/mol; these were consistent with published data under similar conditions (Nakanishi et al., 1978).

The rate constants of the transition between the exposed and the buried forms of tryptophan residues of HMM were estimated from the rates of the temperature-dependent change of ultraviolet difference spectra (Morita, 1977) following rapid temperature changes (T. Yamada, Y. Ohga, H. Shimizu, M. Nakanishi, and M. Tsuboi, unpublished data). It was found that the transitions are much faster than the time resolution of the present stopped-flow apparatus; i.e., the rate constants of the transition between the exposed and the buried forms are much faster than k_e. Therefore relation 2 is valid in the present hydrogen–deuterium exchange of the tryptophan residues in myosin head.

For tryptophan residues of the fast- and slow-exchanging groups, the apparent equilibrium constant K_{app} can be calculated by means of eq 2. The results are summarized in Table II.

Effects of AMPPNP, ATP, and ADP on the Hydrogen–Deuterium Exchange of Tryptophan Residues in HMM. Similar experiments to those described above were performed for HMM solutions to which AMPPNP, ATP, and ADP were added. For the HMM + AMPPNP and HMM + ADP systems, AMPPNP and ADP had been respectively mixed with HMM in H_2O solution prior to mixing with the D_2O solution. For the HMM + ATP system, ATP had been added to the D_2O solution, and then it was mixed with HMM in H_2O solution.

By use of the dissociation constant between AMPPNP and HMM (Yount et al., 1971) and that between ADP and HMM (Arata et al., 1975; Marsh et al., 1978), the amounts of M·AMPPNP and M·ADP complexes were estimated to be over 0.94 per myosin head for the HMM + AMPPNP and HMM + ADP systems. Therefore the molecular species that dominantly contribute to the hydrogen–deuterium exchange reaction are the M·AMPPNP and M·ADP complexes for the HMM + AMPPNP and HMM + ADP systems, respectively. For the HMM + ATP system, the dominant molecular species is the M·ADP·P_i complex; the amount of M·ADP·P_i complex per myosin head is estimated to be about 0.9 on the basis of the dissociation constant between HMM and ATP (Goody et al., 1977), the equilibrium constant between the M·ATP and M·ADP·P_i complexes (Bagshaw & Trentham, 1973; Wolcott & Boyer, 1974; Arata et al., 1975), and the rate of the decomposition of the M·ADP·P_i complex (about 0.01 s^{-1}) under the present experimental conditions.

It should be mentioned that the formation of M·ATP and M·ADP·P_i complexes also induces time-dependent ultraviolet absorption changes in the HMM + ATP system (Morita & Ishigami, 1977). In fact, below ~15 °C, the absorption change due to the formation of M·ATP and M·ADP·P_i was found to superpose, within about 1 s after the mixing of HMM in H_2O solution and ATP in the D_2O solution, on the absorption change caused by the hydrogen to deuterium exchange of the tryptophan residues. Because of this, we could analyze the exchange process only after significant correction for this extra absorption change. Above ~20 °C, however, the absorption change due to the formation of M·ATP and M·ADP·P_i complexes becomes much faster than that of the hydrogen–deuterium exchange of the tryptophan residues in HMM, and so we could analyze the hydrogen–deuterium exchange process without correction for the absorption change due to the formation of M·ATP and M·ADP·P_i.

The observed ultraviolet absorption changes are also included in Figure 1 for the systems HMM + AMPPNP, HMM + ATP, and HMM + ADP. Replots of the data, which are illustrated also in Figure 2, show that the hydrogen–deuterium exchanges are composed of two first-order processes having high and low rates as in the case of free HMM. Furthermore, both the fast- and slow-exchange groups involve about three tryptophan residues per myosin head. The exchange rates were obtained at different temperatures, as shown in Table I for the fast- and slow-exchange groups. The values of the apparent equilibrium constant between the exposed and the buried forms of myosin head calculated by using eq 2 are given in Table II.

Hydrogen–Deuterium Exchange of Tryptophan Residues in SF-1. A similar time-dependent decrease of ultraviolet absorption was observed when HMM was replaced by SF-1. The experimental records of the absorption change were almost

Table III: Rates of Hydrogen–Deuterium Exchange of the Fast-Exchanging and the Slow-Exchanging Groups of the Tryptophan Residues of HMM(try), HMM(chy), and SF-1 at 25 °C[a]

	rate of hydrogen–deuterium exchange (s^{-1})					
	fast-exchanging group			slow-exchanging group		
	HMM(try)	HMM(chy)	SF-1	HMM(try)	HMM(chy)	SF-1
free	2.98	2.13	2.31	0.31	0.28	0.27
+AMPPNP	1.93	0.83		0.35	0.35	
+ATP	1.63	1.01	1.15	0.05	0.06	0.07
+ADP	2.31	1.65	1.73	0.23	0.23	0.25

[a] The conditions are given in the legend to Figure 1.

the same as those in the corresponding cases of HMM if the concentration of the myosin head was equal. The total absorption change measured at several different wavelengths was analyzed and gave a similar difference spectrum to the case of HMM shown in Figure 3. The size of the absorption change of the difference spectrum for the case of SF-1 was slightly smaller (by about 10%) than that for the case of HMM. Therefore the observed absorption change is caused by the hydrogen–deuterium exchange of the tryptophan residues in SF-1. As in the case of HMM, the whole exchange process is composed of two first-order processes with a fast-exchanging and a slow-exchanging group, each involving about three tryptophan residues per myosin head.

Next, we investigated the effects of ATP and ADP on the exchange reaction. The rate of the exchange process at 25 °C decreased in the same order as in the cases of HMM, that is, free SF-1, SF-1 + ADP, and SF-1 + ATP.

It should be mentioned that slight but meaningful differences can be noted in the hydrogen–deuterium exchange process for SF-1 in the corresponding cases of HMM, as shown in Figure 4. These differences were found to be due mainly to the changes in the exchange rate of the tryptophan residues of the fast-exchanging group, as shown in Table III.

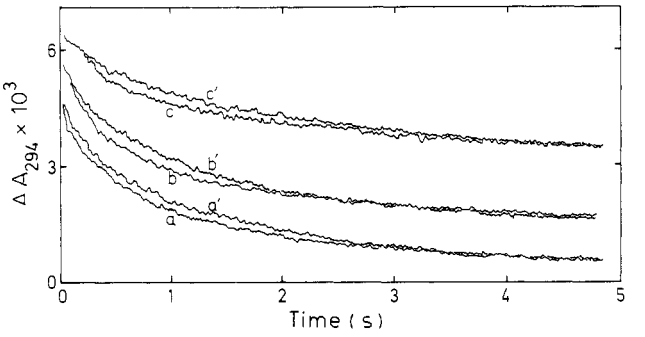


FIGURE 4: Time dependence of the decrease in absorbance at 294 nm observed when (a) HMM in H_2O solution is mixed with D_2O solution, (a') SF-1 in H_2O solution is mixed with D_2O solution, (b) HMM + ADP in H_2O solution is mixed with D_2O solution, (b') SF-1 + ADP in H_2O solution is mixed with D_2O solution, (c) HMM in H_2O solution is mixed with ATP in D_2O solution, and (c') SF-1 in H_2O solution is mixed with ATP in D_2O solution. Conditions: 0.1 M KCl, 2 mM $MgCl_2$, and 20 mM Tris-HCl at pH 8.0 and 25 °C. The final concentrations of HMM and SF-1 were 13 and 29 μM, respectively, and of ATP and ADP, 200 μM. For clarity, the experimental records of M + ADP and M + ATP systems are vertically shifted by 0.9×10^{-3} and 1.8×10^{-3} OD unit, respectively.

Discussion

The present results show that the ultraviolet absorption change observed at around 292 nm is caused by hydrogen–deuterium exchange of the tryptophan residues in HMM and SF-1. As HMM and SF-1 show almost the same time-dependent absorption change, the tryptophan residues giving rise to the absorption change must be localized in the myosin head. This is to be expected since subfragment-2 contains no tryptophan (Lowey et al., 1969). The number of accessible tryptophan residues is estimated to be about seven per myosin head. This is quite compatible with the data reported for the tryptophan content of myosin active fragments, 8.8 (Shimizu et al., 1971) and 7.7 (Shimizu et al., 1971) tryptophan residues per myosin head for HMM and SF-1, respectively, which were estimated by spectrophotometric analysis. Since alkali light chains and DTNB light chains have no tryptophan residues (Frank & Weeds, 1974) and only one (Collins, 1976; Matsuda et al., 1977) tryptophan residue per molecule, respectively, the tryptophan residues detected by the hydrogen–deuterium exchange studies are dominantly located in the heavy chain of the myosin head.

The seven tryptophan residues in the myosin head can be classified into the fast-exchanging group, which is called Trp(f), involving about three residues having an exchange rate of the order of 1 s^{-1}, and the slow-exchanging group, which is called Trp(s), having an exchange rate of the order of 0.1 s^{-1}. These exchange rates are substantially smaller than that of free tryptophan (about 32 s^{-1} at 20 °C, as described under Materials and Methods), indicating that the tryptophan residues are "buried" in the myosin head. We carefully investigated whether some tryptophan residues having the exchange rate of free tryptophan existed, but we could not find any within experimental error. We conclude, therefore, that almost all the tryptophan residues are buried in the molecule.

Recently, nuclear magnetic resonance studies of myosin head have been reported which suggest that myosin head is composed of a rigid core and a flexible domain(s) (Akasaka et al., 1978; Highsmith et al., 1979). The magnitude of apparent equilibrium constant between the exposed and the buried forms around the tryptophan residues as shown in Table II ($K_{app} = 10^{-4} \sim 10^{-1}$) is substantially larger than that around the peptide reported in our previous study ($K_{app} = 10^{-6}$) (Yamada et al., 1977). From differences in the values of apparent equilibrium constants, it is almost certain that the peptides are located in the rigid core of myosin head and the tryptophan residues near the surface.

Moreover, the analyses above employing eq 2 show that the apparent equilibrium constant between the exposed and the buried forms around the tryptophan residues decreases as the temperature is increased for all systems examined (see Table II). This indicates that the local structure around the tryptophan residues is stabilized dominantly by an entropy force; the tryptophan residues are located in hydrophobic domains. Haugland (1975) and Bagshaw & Reed (1976) have suggested that the active site of myosin consists of a large hydrophobic crevice. We may assume that the tryptophan residues are buried in the crevice of the active site of myosin head.

As shown under Results, the overall exchange rate of the seven tryptophan residues is substantially decreased by the addition of ATP. This indicates that the conformation of the myosin head changes by forming the M·ADP·P_i intermediate complex so as to make almost all the seven tryptophan residues

become more buried. This conclusion is qualitatively consistent with those obtained by the studies of ultraviolet absorption (Morita, 1967) and fluorescence (Werber et al., 1972); addition of ATP (and ADP) makes tryptophan residues (by about two residues per myosin head) become more buried in the myosin head. However, the present results indicate that the conformational changes of myosin head during ATP hydrolysis alter the environment of many more tryptophan residues than considered so far. We therefore conclude that the ATP hydrolysis at the active site of myosin head induces conformational changes of wide domains in myosin head.

It has been suggested that tryptophan residues are located near the adenine moiety of ATP bound to the myosin head (Murphy, 1974; Onishi et al., 1977). If this is the case, the hydrogen–deuterium exchange rate of the tryptophan residue(s), i.e., the local conformation around the tryptophan residue(s), may change on the binding of nucleotides with myosin head. The apparent equilibrium constants between the exposed and the buried forms around the tryptophan residues in the Trp(f) group change roughly in a similar fashion by the additions of AMPPNP, ATP, and ADP, as can be seen in Table II. On the other hand, the apparent equilibrium constants between the exposed and the buried forms around the tryptophan residues in the Trp(s) group change in a different way on the addition of AMPPNP, ATP, and ADP; it is exceptionally strongly decreased in the case of the addition of ATP. These results are consistent with a view that the Trp(f) domain(s) is (are) located near the adenine moiety of ATP which is bound to the myosin active site, and the Trp(s) domain(s) is spatially distinct from this domain.

The exchange rates of the tryptophan residues of HMM(try) for the cases of free HMM, HMM + AMPPNP, HMM + ATP, and HMM + ADP are slightly higher than those of the corresponding cases of SF-1. As can be seen in Table III, the differences are due mainly to the changes of the exchange rates of the Trp(f) group. The pattern of $NaDodSO_4$–polyacrylamide gel electrophoresis indicates that the heavy chains of HMM(try) are split at several parts of the peptide bonds by the proteolytic digestion while those of SF-1 remain intact (Hayashi, 1972; Weeds & Taylor, 1975). We may reasonably assume that the higher exchange rates of the Trp(f) group of HMM(try) are due to the damage in the heavy chains caused by the tryptic digestion. As trypsin probably splits the peptide bonds at the surface of the myosin head, this assumption is consistent with the conclusion above that the Trp(f) domain is located near the surface of the myosin head. Very recently it was found that the heavy chain of myosin head is split into several fragments by tryptic digestion and that the two fragments of heavy chain (27K and 50K fragments) contain about two tryptophan residues per mole (T. Hozumi, personal communication).

The above discussions lead us to consider that the ATPase activity might be different between HMM(try) and SF-1. However, no significant difference has been observed in the ATPase activity between HMM(try) and SF-1 (Taylor, 1977; Margossian & Lowey, 1978). Since the myosin head can bind inorganic pyrophosphate (Tonomura & Morita, 1959) and ribose 5′-triphosphate (Asakawa et al., 1978), nucleotides seem to be anchored to the myosin active site dominantly by the interaction between the phosphate parts of the nucleotides and the active site of the myosin head. Thus we may speculate that, as long as the sites which bind the phosphate parts of the nucleotides remain intact, the slight damage(s) of the myosin head near the adenine moiety of the bound nucleotide (where the Trp(f) domain may be located) might not induce detectable changes of the ATPase activity.

We performed similar hydrogen–deuterium exchange studies using HMM(chy) at 25 °C for free HMM, HMM + AMPPNP, HMM + ATP, and HMM + ADP systems. The experimental records of the absorption change (data not shown) were almost the same within experimental error as those of the corresponding cases of SF-1 except that the magnitudes of the absorption changes were slightly smaller for the cases of SF-1. The rate constants obtained are included in Table III. The heavy chains are almost intact for HMM(chy) as well as for SF-1 (Weeds & Taylor, 1975), while DTNB light chains remain almost intact for HMM(chy) and are completely depleted for SF-1 (Weeds & Taylor, 1975). We may conclude that almost no tryptophan residue is located in the vicinity of the DTNB light chain binding site of the heavy chain. The decrease in the total absorption change of the hydrogen–deuterium exchange of SF-1 compared with that of HMM may be due to the lack of DTNB light chains for SF-1 (DTNB light chains contain one tryptophan residues per molecule as described above). The above result further indicates that the local conformations around the tryptophan residues do not differ significantly whether ATP hydrolysis takes place at two heads or a single head. Thus the present results provide no evidence of the interaction of two heads of myosin during ATP hydrolysis.

Next, let us compare the conformational changes around the tryptophan residues shown in the present studies and those around the peptides part shown in our previous study (Yamada et al., 1977). The hydrogen–deuterium exchange of the tryptophan is slower at 25 °C in the system of M + ATP than that of M + ADP, as can be seen in Figure 1. On the other hand, the exchange rate of the peptide hydrogen is faster in the system of M + ATP than that of M + ADP [Figure 4, Yamada et al. (1977)]. These results indicate that the exchange of tryptophan becomes faster at the reaction step of M·ADP·P_i (M + ATP system) to M·ADP + P_i (M + ADP system) while that of peptide becomes slower. A similar reversl of the hydrogen–deuterium exchange can be noticed at 15 °C compared to that at 25 °C between the tryptophan domains (data not shown) and the peptide domain [Figure 3, Yamada et al. (1977)]. These results indicate that the tryptophan residues become more exposed while the peptide part becomes more buried at the reaction step of M·ADP·P_i to M·ADP + P_i (step b, Scheme I). Since the helix content does not change appreciably during ATP hydrolysis (Tonomura et al., 1963; Gratzer & Lowey, 1969; Yamada et al., 1977), we may conclude that these conformational changes take place at the localized domains of the myosin head. Therefore these findings strongly suggest that the conformation of myosin head changes in a reciprocal way between the spatially distinct domains at the reaction step M·ADP·P_i to M·ADP + P_i.

We may consider that these structural changes of myosin head taking place in a reciprocal way during ATP splitting play some role in the dissociation and reassociation of the myosin head and actin filament and in the motion of the myosin head on actin filament during muscle contraction. This is consistent with the view that myosin head correlatively changes the conformation between ATP binding site and actin binding site during ATP hydrolysis (Shimizu & Yamada, 1975; Morales & Botts, 1979).

We have to keep in mind that some of the above discussions assumed that the hydrogen–deuterium exchange of the seven tryptophan residues of the myosin head involves only two groups. The seven tryptophan residues are possibly located at spatially distinct domains of the myosin head and accord-

ingly have different exchange rates. Therefore the obtained exchange rates of the two tryptophan groups may be merely averaged ones.

In any case, more work is required to confirm the above conclusions.

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