BBA 45813

INTERACTION OF HEAVY MEROMYOSIN WITH SUBSTRATE

III. DIFFERENCE SPECTRUM OF ULTRAVIOLET ABSORPTION IN SUBFRAGMENT 1 INDUCED BY ATP OR ADP

FUMI MORITA AND TAKIKO SHIMIZU

Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo (Japan)

(Received February 7th, 1969)

SUMMARY

1. The ultraviolet absorption difference spectrum of Subfragment 1 induced by ATP was measured in the presence of $MgCl_2$. The shape of the difference spectrum was the same as that of heavy meromyosin. The difference molar extinction coefficient at the major peak, $\Delta E_{289\ m\mu}$, was $2200 \pm 400\ M^{-1} \cdot cm^{-1}$, which was 44% of that of heavy meromyosin. It decreased with time especially near 290 mμ and reached an identical shape as that induced by ADP.

2. The difference spectra of Subfragment 1 induced by the addition of ADP and of heavy meromyosin had identical forms. $\Delta E_{288\ m\mu}$ was $1300 \pm 400\ M^{-1} \cdot cm^{-1}$, which was 52% of the value of heavy meromyosin.

3. The dependence on the concentration of the difference absorbance induced by ADP was measured at 288 mμ. A strong binding of ADP to Subfragment 1 was suggested. The minimum ADP concentration needed to give the maximum change in the difference absorbance was 1.2 times the concentration of Subfragment 1.

4. Both difference spectra due to ATP and ADP were not dependent on the KCl concentration.

5. Decay of the difference spectrum of Subfragment 1 induced by ATP was measured. The value of k_8 obtained from Chance's equation agreed with the maximum velocity of steady-state ATPase.

INTRODUCTION

The ultraviolet absorption difference spectrum due to aromatic chromophores in heavy meromyosin was previously shown to be induced by the addition of ATP or by its analogs[1,2]. It was shown to be a reflection of a local structural change near the active site of heavy meromyosin by the binding of the substrate. In the preceding paper[3], it was demonstrated by the stopped flow measurement that, in the presence of $MgCl_2$, the difference spectrum occurs associating with the Michaelis–Menten complex which is formed at one of the two binding sites of ATP in heavy meromyosin.

There have been many studies and discussions about the subunit structure of myosin and of heavy meromyosin mainly on the basis of hydrodynamic measurements[4–10]. Recent electron microscopic investigations corroborate the proposal that

a subunit structure in the globular head of the heavy meromyosin exists[11,12]. However, the correlation between the subunit structure of heavy meromyosin and the binding sites of the substrate is still speculative. Thus, it is important to study the characteristics of the active subfragment called Subfragment 1, first isolated by MULLER AND PERRY[6], whose molecular weight is nearly one third of that of heavy meromyosin.

In order to compare them with those of heavy meromyosin, an attempt to obtain the ultraviolet absorption difference spectra of Subfragment 1 induced by the addition of ATP or ADP in the presence of $MgCl_2$ was made. The shape and characteristics of the difference spectra of Subfragment 1 and of heavy meromyosin were identical, but the difference molar extinction coefficients, ΔE, of Subfragment 1 were about one half those of heavy meromyosin. Data suggest that Subfragment 1 preparation is an equimolar mixture of two kinds of particles differing with respect to the spectral change by the binding of the substrate.

EXPERIMENTAL

The preparation of Subfragment 1 was made by the method described by YAGI AND YAZAWA[13]. The ultraviolet absorption difference spectrum of Subfragment 1 was measured using the double cell method[14] which was the same procedure using a Hitachi Recording Spectrophotometer type EPS-3T for measuring heavy meromyosin. ATPase activity at the steady-state was determined by measuring the time-course of P_i liberation. The concentration of P_i was determined by the method of FISKE AND SUBBAROW[15]. All experiments were effected at 25° in the presence of 10 mM $MgCl_2$ and 50 mM Tris–HCl buffer at pH 8. The concentration of Subfragment 1 was estimated from the absorption at 280 mμ using the value of $E^{1\%}_{1\,cm}$ of 7.9. ATP, ADP, trypsin and a trypsin inhibitor were purchased from Sigma Chemical Co. Rate constants and molar difference extinction coefficients were calculated, basing the value of $1.3 \cdot 10^5$ as the molecular weight of Subfragment 1 (refs. 8–10, 13).

RESULTS

The ultraviolet absorption difference spectrum between the Subfragment 1 solutions with and without ATP has been measured. Fig. 1 represents the difference spectrum. Curve a is the trace obtained immediately after the addition of ATP and shows two positive peaks: a major one at 289 mμ and a minor one at 281 mμ. Near 300 mμ exists a shoulder whose whole curve is identical with that of heavy meromyosin induced by ATP[2]. The molar difference extinction coefficient at the major peak, $\Delta E_{289\ m\mu}$, was $2200 \pm 400\ M^{-1} \cdot cm^{-1}$ (15 determinations) in the presence of sufficient ATP. This is 44% of the value of heavy meromyosin[2]. Both difference spectra near 290 mμ decreased with time and reached the difference spectrum as shown in Curve d in Fig. 1. As shown in Fig. 2, this curve has two positive peaks at 288 and 280 mμ as does the curve representing the addition of ADP. The shape of the curve is identical with that of the difference spectrum of heavy meromyosin induced by ADP[2]. The molar difference extinction coefficient at 288 mμ, $\Delta E_{288\ m\mu}$, was $1300 \pm 400\ M^{-1} \cdot cm^{-1}$ (41 determinations) in the presence of sufficient ADP. This is 52% of the value of heavy meromyosin[2].

The difference absorbance at 288 mμ, $\Delta A_{288\ m\mu}$, induced by the addition o

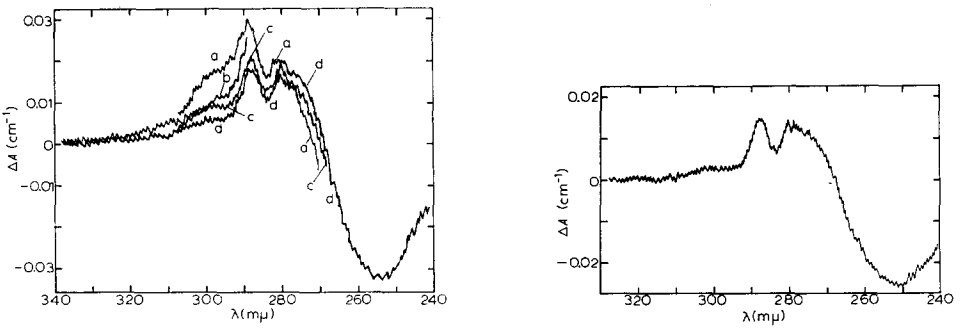

Fig. 1. Difference spectrum of Subfragment 1 after the addition of ATP. Experimental condition was 1.88 mg of Subfragment 1 per ml, 0.12 M KCl, and 70 μM ATP. The time after adding ATP to Subfragment 1 at which the wavelength of 289 mμ was reached was 30, 71, 134 and 551 sec for Curves a, b, c and d, respectively.

Fig. 2. Difference spectrum of Subfragment 1 induced by the addition of ADP. Experimental condition was 1.86 mg Subfragment 1 per ml, 64 mM KCl and 0.1 mM ADP.

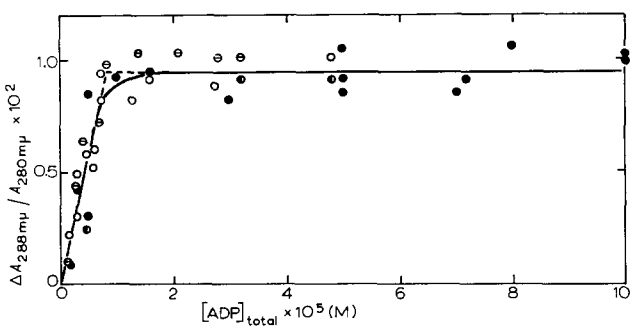

Fig. 3. Dependence of $\Delta A_{288\,m\mu}$ on the concentration of ADP in the presence of 0.06 M KCl. $\Delta A_{288\,m\mu}/A_{280\,m\mu}$ in ordinate is defined as the ratio of the difference absorbance at 288 mμ, $\Delta A_{288\,m\mu}$, and the absorbancy of the Subfragment 1 solution at 280 mμ, $A_{280\,m\mu}$. Concentration of Subfragment 1 ranged from 1.86 to 2.23 mg/ml. Different symbol shows different preparation.

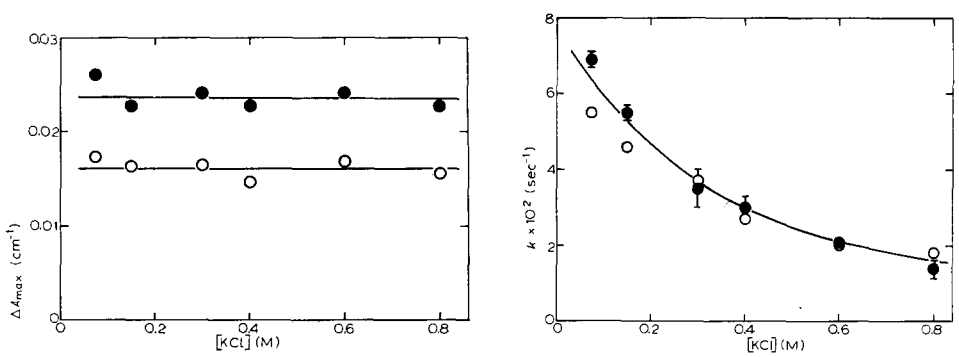

Fig. 4. Effect of KCl on ΔA. 1.30 mg Subfragment 1 per ml was used. A_{max} is the difference absorbance at 289 mμ due to ATP (●) and at 288 mμ due to ADP (○) which was obtained after the addition of 57.1 μM ATP.

Fig. 5. Comparison of k_s and k_2. For the determination of k_s, 1.30 mg Subfragment 1 per ml, and 57.1 μM ATP were used. For the determination of k_2, 0.65 or 0.99 mg Subfragment 1 per ml, and 0.83 or 0.87 mM ATP were used. k_s (●) is the average with the standard deviation of 4 determinations and k_2 (○) is the average of 2 determinations.

ADP was measured as a function of the ADP concentration, as shown in Fig. 3. Data suggest a strong binding of ADP to Subfragment 1 as is the case of heavy meromyosin[2]. The minimum concentration of ADP needed to give the maximum $\Delta A_{288\ m\mu}$ was 8.4 μM when $\Delta A_{288\ m\mu}/A_{280\ m\mu}$ was used as the ordinate (Fig. 3). This concentration is 1.2 times the concentration of Subfragment 1 whose absorbance at 280 mμ is 1 cm^{-1}.

The difference spectrum of Subfragment 1 which was due to the presence of a sufficient concentration of ATP or ADP was independent of the KCl concentration, as seen in Fig. 4. The same tendency also has been seen in heavy meromyosin (F. MORITA, unpublished results).

Decay of the difference spectra of Subfragment 1 induced by ATP and of heavy meromyosin were similarly measured at the fixed wavelength of 293 mμ. Data were analyzed by the equation of CHANCE[16] using the half-life of the difference absorbance and the initial concentration of ATP and of Subfragment 1 (refs. 2, 3). The calculated values of k_s are shown in Fig. 5. As shown in Fig. 5, the V/e (k_2) values also were determined from the initial velocity of the ATPase reaction at the steady state. Both k_s and k_2 values agree within a wide range in the KCl concentration.

DISCUSSION

As shown in Figs. 1 and 2, the shape of the difference spectra of Subfragment 1 induced by ATP and by ADP were identical with those of heavy meromyosin. This suggests that the higher-order structure of Subfragment 1 around the active site responsible for the spectral change is preserved when it is prepared from heavy meromyosin. Furthermore, the difference spectra were independent of the KCl concentration (Fig. 4), and k_s and k_2 values were in agreement (Fig. 5). These characteristics and those of heavy meromyosin are identical. The only variation in the difference spectra of both proteins is the reduction in ΔE values of Subfragment 1 to one-half of those of heavy meromyosin. Therefore, the reduction in ΔE values appears to be due to the original molecular structure of heavy meromyosin and not to a damage around the active site with the preparation of Subfragment 1.

By directly measuring the binding it has been shown that 2 moles of ATP or its analogs are bound per mole of myosin or heavy meromyosin[17–19] and that 1 mole is bound per mole of Subfragment 1 (refs. 18, 20). The dependence on the concentration of the substrate[2,3] of the change in ultraviolet absorption of heavy meromyosin has been studied. By using the stopped flow method, the minimum concentration of ATP needed to give the maximum change in ΔA was found to be twice the concentration of heavy meromyosin which was used[3]. On the other hand, when ADP was employed as the substrate, the minimum concentration of ADP was equal to the concentration of heavy meromyosin[2]. These results suggest that the two binding sites in heavy meromyosin are heterogeneous with respect to the spectral change by the substrate. The apparent differences in the equivalent concentration of substrate needed to give the maximum spectral change may be due to the differences in the mutual binding constants of the substrate to the two binding sites under various conditions. Furthermore, as described in the preceding paper[3], the kinetic study of the spectral change has shown that only one of the two ATP binding sites works in the apparent steady-state ATPase accompanying the ultraviolet difference spectrum on its Michaelis–Menten complex.

The following conclusion that the reduction of ΔE values in Subfragment 1 to one-half those in heavy meromyosin is substantiated if it is accepted that there is one binding site of substrate per subfragment particle, that there are 2 sites in 1 heavy meromyosin and that only one of the two sites concern the spectral change by the binding of the substrate. Furthermore, the Subfragment 1 preparation must be heterogeneous with respect to the spectral change by the substrate as it contains two kinds of particles with the same molar ratio; one type of Subfragment 1 particle and not the other contains the site accompanying the spectral change by the binding of substrate. The binding site of the substrate not accompanying the spectral change may locate either on the same Subfragment 1 particle containing the site, which accompanies the spectral change, or on the other particle. Nevertheless, the two types of Subfragment 1 particles must have a similar size and shape because the Subfragment 1 preparation is hydrodynamically[6,9,13] homogeneous. Satisfying such criteria, there are several methods of deriving Subfragment 1 from heavy meromyosin, however, no unique answer has been obtained.

As shown in Fig. 3, the minimum concentration of ADP needed to give the maximum change in absorbance was almost equal to the concentration of Subfragment 1. When heavy meromyosin was used, a critical ADP concentration also was equal to the concentration of heavy meromyosin[2]. Therefore, the binding of ADP to the site accompanying the spectral change must be stronger than to the other site in heavy meromyosin; however, they are nearly equal in Subfragment 1. Affinities of the two sites in myosin or in heavy meromyosin to substrates are so similar that they are almost indistinguishable by equilibrium dialysis[17] or by gel filtration[19]. Therefore, only a minor change in the affinity of either of the two sites may produce an apparent difference between heavy meromyosin and Subfragment 1, which may occur during the preparation of Subfragment 1.

ACKNOWLEDGMENTS

We are grateful to Dr. K. Yagi for his discussions and support. This work was supported by a grant from the Muscular Dystrophy Association of America.

REFERENCES

1 F. MORITA AND K. YAGI, *Biochem. Biophys. Res. Commun.*, 22 (1966) 297.
2 F. MORITA, *J. Biol. Chem.*, 242 (1967) 4501.
3 F. MORITA, *Biochim. Biophys. Acta*, 172 (1969) 319.
4 W. W. KIELLY AND W. F. HARRINGTON, *Biochim. Biophys. Acta*, 41 (1960) 401.
5 P. A. SMALL, W. F. HARRINGTON AND W. W. KIELLEY, *Biochim. Biophys. Acta*, 49 (1961) 462.
6 H. MUELLER AND S. V. PERRY, *Biochem. J.*, 85 (1962) 431.
7 D. M. YOUNG, S. HIMMELFARB AND W. F. HARRINGTON, *J. Biol. Chem.*, 293 (1964) 2822.
8 H. MUELLER, *J. Biol. Chem.*, 240 (1965) 3816.
9 M. YOUNG, S. HIMMELFARB AND W. F. HARRINGTON, *J. Biol. Chem.*, 240 (1965) 2428.
10 J. M. JONES AND S. V. PERRY, *Biochem. J.*, 100 (1966) 120.
11 C. R. ZOBEL, *Biochim. Biophys. Acta*, 140 (1967) 222.
12 H. S. SLAYTER AND S. LOWEY, *Proc. Natl. Acad. Sci. U.S.*, 58 (1967) 1611.
13 K. YAGI AND Y. YAZAWA, *J. Biochem. Tokyo*, 60 (1966) 450.
14 T. T. HERSKOVITS AND M. LASKOWSKI, JR., *J. Biol. Chem.*, 237 (1962) 3418.
15 C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1952) 275.
16 B. CHANCE, *J. Biol. Chem.*, 151 (1943) 553.
17 Y. TONOMURA AND F. MORITA, *J. Biochem. Tokyo*, 46 (1959) 1367.
18 K. M. NAUSS AND J. GERGELY, *Federation Proc.*, 26 (1967) 2625.
19 L. H. SCHLISELFELD AND M. BÁRÁNY, *Biochemistry*, 7 (1968) 3206.
20 M. YOUNG, *J. Biol. Chem.*, 242 (1967) 2790.