Temperature-induced ultraviolet absorption changes of heavy meromyosin

An application of a computerized spectrophotometer system

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The UV absorption of HMM (heavy meromyosin) was measured at various temperatures with a computerized spectrophotometer system. HMM showed temperature-induced absorption changes in the presence and absence of nucleotides. The temperature-induced absorption change at 293 nm, which is due to conformational changes around the tryptophan residues of HMM, was enhanced in the presence of nucleotides. The temperature-induced difference spectra of HMM + AMPPNP relative to HMM obtained by using a conventional spectrophotometer [(1977) J. Biochem. (Tokyo) 81, 313–320] could be reproduced by subtracting the temperature-induced spectral changes of HMM from those of HMM + AMPPNP.

Myosin *ATPase* *Ultraviolet spectrophotometry* *Computer data processing*

1. INTRODUCTION

Myosin head shows conformational changes during the hydrolysis of ATP [1–3]. The author in [4] has studied the difference UV absorption spectra of HMM + AMPPNP relative to HMM at various temperatures by using a conventional spectrophotometer, and reported that the HMM·AMPPNP complex shows temperature-induced absorption changes.

Authors in [5] recently found that the hydrogen to deuterium exchange rates of tryptophan residues of HMM show temperature-induced changes in the absence as well as in the presence of nucleotides. Since a conventional spectrophotometer uses the absorption of HMM as a reference to obtain the difference spectra of the HMM + AMPPNP system, temperature-induced spectral changes of HMM itself may be overlooked.

We measured here the UV absorption of HMM in the presence and absence of nucleotides at various temperatures with a computerized spectrophotometer system which had a high signal-to-noise ratio, high density of data points and functions of highly reliable storage and versatile analysis of spectroscopic data [6]. The results indicate that not only HMM + nucleotide complexes but also HMM itself shows temperature-induced UV absorption changes.

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Abbreviations: HMM, heavy meromyosin; AMPPNP, adenylyl 5′-imidodiphosphate; PP_i, pyrophosphate; UV, ultraviolet; Mops, 3-(*N*-morpholino)propane sulfonate

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2. MATERIALS AND METHODS

HMM was prepared by chymotryptic digestion of myosin as in [7] and purified as in [5]. The absorbance of HMM was assumed to be $E^{1\%}_{280} = 6.47$ [8]. The M_r of HMM was assumed to be 340000 [9].

ADP and AMPPNP were purchased from Boehringer Mannheim-Yamanouchi Biochemicals Co. The absorption coefficient of both ADP and AMPPNP was assumed to be $1.54 \times 10^4\ M^{-1} \cdot cm^{-1}$ at 260 nm [10].

Optical absorption of HMM solution was measured with a computerized spectrophotometer system [6] from 250 to 320 nm wavelength at 2-nm interval. The sensitivity of the spectrophotometer was $\pm 0.00015\ A$ unit. Measurements were made between 5 and 25°C at 0.4°C intervals with an accuracy of ~0.01°C by continuously changing the temperature of the HMM solution with a computer-controlled thermoregulator at a rate of 0.8°C/min. The data were stored on floppy disks, analyzed by a computer and printed out accordingly.

3. RESULTS AND DISCUSSION

In fig.1A are shown UV absorption spectra of HMM and HMM + AMPPNP. When the temperature was changed both systems showed small absorption changes. To observe the temperature-induced spectral changes clearly, we calculated the difference absorption spectrum, ABS(25) – ABS(5), by subtracting the spectrum of 5°C, ABS(5), from that of 25°C, ABS(25), for each system.

As shown in fig.1B the difference spectrum of HMM, ABS(25) – ABS(5), had a peak at about 293 nm and a trough at about 281 nm. The spectral changes were characteristic of a red shift of tryptophan absorption. The difference spectra of HMM + AMPPNP, ABS(25) – ABS(5), had a peak at about 293 nm and a deep trough at about 260 nm. The peak at about 293 nm indicated that temperature-dependent structural changes similar to those of free HMM also took place around tryptophan residues of nucleotide-bound myosin head. This suggests that tryptophan residues of HMM become more buried in the absence as well as in the presence of nucleotides as the temperature is

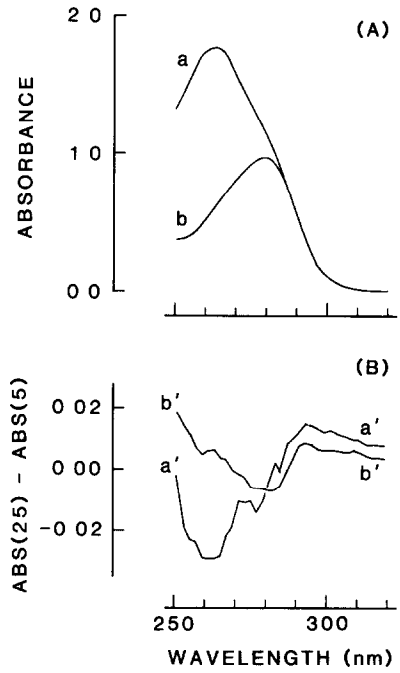

Fig.1. (A) UV absorption spectra of HMM in the presence and absence of AMPPNP at room temperature. (a) HMM + AMPPNP and (b) HMM. The former system has extra absorption due to AMPPNP. (B) Difference spectra obtained by subtracting the absorption spectra at 5°C, ABS(5), from those at 25°C, ABS(25) (a′) HMM + AMPPNP and (b′) HMM Conditions: 1 5 mg/ml HMM, with or without 78 μM AMPPNP, 0.1 M KCl, 5 mM $MgCl_2$, 25 mM Mops (pH 7.0).

elevated. This is in accord with results obtained by a hydrogen to deuterium exchange study of tryptophan residues of HMM [5].

It is noted in fig.1B that there existed a difference between the difference spectrum of HMM + AMPPNP and that of free HMM in the spectral region 250–270 nm. The HMM + ADP system showed temperature-induced difference spectral changes similar to those of the HMM + AMPPNP system while the temperature-induced difference spectral changes of HMM + PP_i resembled those of free HMM. These results suggest that the nucleotide bound to myosin head shows temperature-dependent configurational changes. This is consistent with the temperature-dependent state changes of the nucleotide bound to myosin head observed by ^{31}P NMR spectroscopy [11].

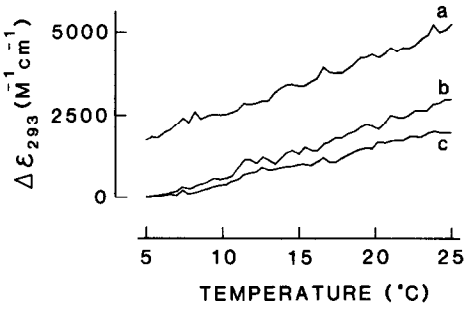

Fig.2. Absorbance at 293 nm of HMM at various temperatures in HMM + AMPPNP, HMM + ADP and free HMM systems relative to that of HMM at 5°C. Firstly, difference absorbance changes at 293 nm, ABS(T) − ABS(5), were plotted as a function of temperature for each system. Then the data for HMM + AMPPNP and HMM + ADP were shifted by taking into consideration the nucleotide-induced absorbance changes of HMM [4]. Each datum is the average of two experiments. (a) HMM + 78 μM AMPPNP, (b) HMM + 80 μM ADP, (c) HMM without nucleotide. Other conditions were the same as those in fig.1.

By calculating difference spectra like those in fig.1B at various temperatures, we could plot absorption changes at 293 nm of HMM as a function of temperature. As shown in fig.2 the slope of the temperature-induced absorbance change at 293 nm became steeper in the order of HMM, HMM + PP_i (not shown), HMM + ADP and HMM + AMPPNP. This indicates that the temperature-induced structural changes around the tryptophan residues become enhanced when myosin head binds nucleotides. This is in accord with results obtained in a study of hydrogen to deuterium exchange of tryptophan residues of HMM [5].

When we subtract the temperature-induced difference spectra of HMM, as shown in fig.1B, from those of HMM + AMPPNP at various temperatures we can obtain temperature-dependent difference spectra which can be compared with those obtained by use of a conventional spectrophotometer [4]. The two data fitted reasonably well with each other.

In conclusion, this study indicates that

(i) Myosin head intrinsically has temperature-dependent structures around the tryptophan residues; and

(ii) The binding of nucleotides to myosin head enhances the temperature-induced structural changes around the tryptophan residues.

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