SPECTRAL SHIFT IN HEAVY-MEROMYOSIN INDUCED BY SUBSTRATE

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Introduction: There are accumulating evidences that the globular enzyme protein induces a conformational transition by the binding of substrate or inhibitor (Havsteen and Hess (1962), Parker and Lumry (1963), Rosenberg, Theorell and Yonetani (1965), Hanlon and Westhead (1965)). In muscle protein, Tonomura et al. have observed a change in b_o term of the optical rotatory dispersion in myosin A or HMM[1] by the addition of ATP or PP (1963a), (1963b), (1965). Iyengar, Glauser and Davies (1964) observed the hyperchromism of the peptide backbone absorption in HMM by the addition of ATP.

It has been established that the absorption spectrum due to $\pi \rightarrow \pi^*$ transition of aromatic chromophore causes a red shift of the absorption band with increase of the refractive index of the environment. Hence the shift of the absorption band in the vicinity of 280 mμ of a protein is regarded as a reflection of a conformational change of the protein near to the side chain chromophore (Yanari and Bovey, 1960).

In this report, it is described that a remarkable red shift of the absorption band in the vicinity of 280 mu of HMM was found by the addition of ATP or its analogs.

Experimental: HMM was prepared from rabbit skeletal muscle by the method similar to that of Szent-Györgyi (1960) and lyophirized in the

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[1] Following abbreviations will be used; HMM: heavy-meromyosin, PP: sodium pyrophosphate, TP: sodium triphosphate.

presence of 0.1 M sucrose. The sample was dissolved in 0.02 M tris-HCl buffer (pH 8.0) containing 0.15 M KCl and dialyzed against the same buffer solution to remove sucrose. After the centrifugation at 10^5 x g for 2 hours, it was purified through a DEAE-cellulose column by the similar method to that of Mueller and Perry (1961). The fraction eluted as a single peak by 0.25 M KCl was used throughout the experiment. The fraction was devoid completely of deaminase activity which interferes the measurement of absorption spectrum.

Difference spectrum measurement were carried out in a Hitachi Recording Spectrophotometer type EPS-2. The cell was maintained at a constant temperature (25°) by means of circulating water through a jacket. To cancel out the contribution of ATP or ADP to the difference spectrum, a pair of matched double cells which are analogous to that for the solvent purturbation technique by Herskovits and Laskowski (1962) was used. The light path length was 4 mm for each compartment. In the reference cell, HMM and ATP containing the same solvent constituent were separately placed in the each compartment. In the sample cell, the mixture of HMM and ATP containing the same solvent as that used in the reference was placed in one compartment and only solvent in the other. The recording could be begun about one minute after HMM and ATP were mixed. Concentration of HMM was determined spectrophotometrically by measuring the optical density at 280 mμ and calculating of the concentration on the basis that $E_{1\%}^{1cm}$ at 280 mμ is 6.25 (Yazawa, private communication). Sigma ATP and Boehringer & Soehne ADP (sodium salt) were used without further purification.

Results: The difference spectra of HMM induced by the addition of ATP are shown in Fig. 1. Two maxima at around 280 and 288 mμ were observed. The difference decreased with the decrease of ATP concentration.

Dependence of the difference in absorption at 288 mμ (ΔE_{288}) on the added concentration of ATP is shown in Fig. 2. The ΔE_{288} induced by

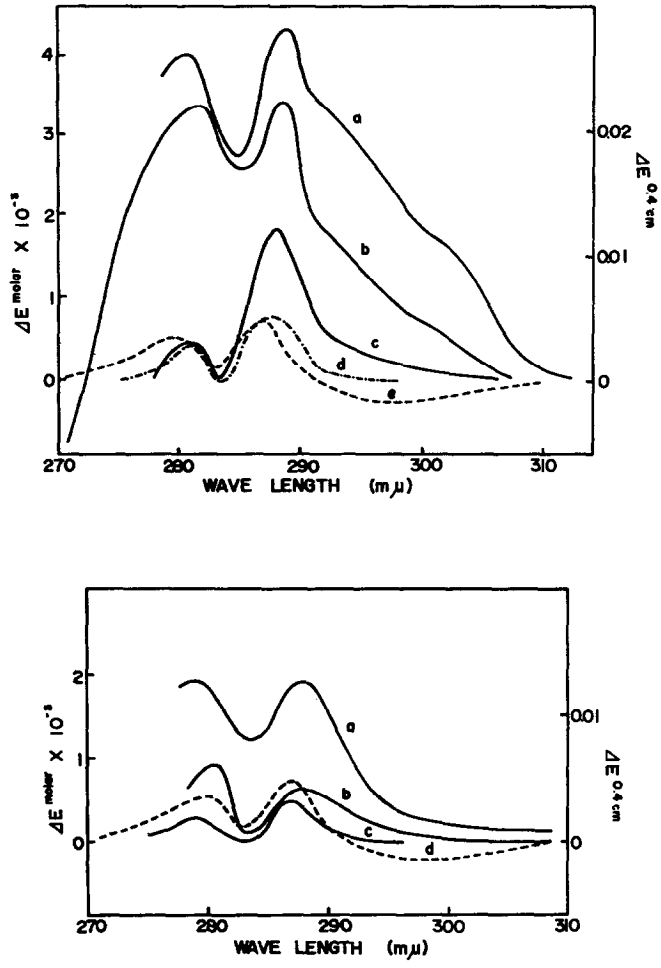

Fig. 1. Difference spectrum produced by the addition of substrate

A: The difference spectrum was produced by the addition of 5.6×10^{-4} M ATP (a), 2.95×10^{-4} M ATP (b), 5.6×10^{-4} M ADP (c), 5.9×10^{-3} M PP (e) in the presence of 5.9×10^{-4} M $MgCl_2$, 4.9 mg/ml HMM, 0.22 M KCl, 0.018 M tris-HCl buffer pH 8.2; 5.9×10^{-3} M TP (d) in the presence of 5.9×10^{-4} M $MgCl_2$, 5.1 mg/ml, 0.11 M KCl, 0.084 M tris-HCl buffer pH 8.2. B: The difference spectrum was produced by the addition of 5.6×10^{-4} M ATP (a), 5.6×10^{-4} M ADP (b), 5.9×10^{-3} M PP (d) in the presence of 5.9×10^{-4} M $CaCl_2$, 4.9 mg/ml HMM, 0.22 M KCl, 0.018 M tris-HCl buffer pH 8.2; the difference spectrum was produced by the addition of 5.9×10^{-3} M TP (c) in the presence of 5.9×10^{-4} M $CaCl_2$, 5.1 mg/ml HMM, 0.11 M KCl, 0.084 M tris-HCl buffer pH 8.2. ΔE^{molar} was calculated assuming that the molecular weight of HMM is 3.62×10^5.

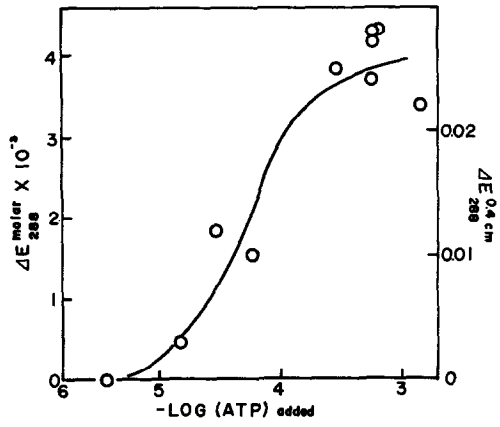

Fig. 2. Effect of ATP concentration on ΔE_{288}

In the presence of 4.9 mg/ml HMM, 5.9 x 10^{-4} M $MgCl_2$, 0.22 M KCl and 0.018 M tris-HCl buffer pH 8.2. ΔE^{molar} was calculated assuming that the molecular weight of HMM is 3.62 x 10^5.

4 x 10^{-4} M ATP in the presence of 9.4 x 10^{-4} M magnesium chloride obeyed the Lambert-Beer's relation in the range of HMM concentration from 2 to 6.4 mg/ml. As shown in Fig. 1, analogs of ATP induced a very little difference spectrum which was similar shape to those by ATP. The effect was in the order of ATP > ADP > TP ~ PP. After allowing the sample to stand for several hours at 25°, the difference spectrum induced by ATP was found to be that of ADP-level. As shown in Fig. 1B, the difference was smaller in the presence of calcium chloride,

Table I

Effect of pH on ΔE_{288}

pH	Buffer	Concentration of KCl (M)	$\Delta E_{288}^{0.4\ cm}$
6.12	0.077 M Phosphate	0.11	0.028
7.03	0.077 M Phosphate	0.11	0.030
8.15	0.077 M Phosphate	0.11	0.034
8.20	0.018 M Tris-HCl	0.22	0.029~0.025
8.95	0.077 M Phosphate	0.11	0.026

The difference spectrum was produced by the addition of 5.6 x 10^{-4} M ATP in the presence of 5.1 mg/ml HMM and 5.9 x 10^{-4} M $MgCl_2$.

especially with ATP or ADP than in the presence of magnesium chloride. The effect of pH on ΔE_{288} induced by ATP is represented in Table I. The result indicated that the ΔE_{288} were almost independent of pH in the range from 6 to 9.

Discussion: There was no detectable peak of tryptophan difference spectrum around 293 mμ in this experiment. The difference spectrum observed here can be ascribable mainly to a red shift of the band due to the side chain chromophores in tyrosine residues. Assuming that only tyrosine chromophores contribute to the peak at 288 mμ, the difference extinction coefficient per residue at 5×10^{-4} M ATP in the presence of magnesium chloride is 55 using the value of 76 for the tyrosine residues per mole of HMM (Kominz, Hough, Symonds and Laki, 1954). This is fairly smaller than the value of several hundreds observed in a blue shift by the denaturation of globular proteins (Yanari and Bovey, 1960) and even that by acid denaturation of HMM (Morita, unpublished observation). We interpret the red shift observed here, to mean that a conformational change was induced in HMM molecule by the binding of substrate to the active site of ATPase (E.C.3.6.1.3.) and several tyrosine chromophores in the vicinity of the active site were buried into the interior of the protein moiety. It has been reported that the helical content of HMM was decreased by the addition of ATP (Iyengar, Glauser and Davies (1964), Sekiya, Mii, Takeuchi and Tonomura (1965)). Doty and Gratzer (1962) observed a blue shift of the adsorption band around 280 mμ in the tyrosine glutamic acid copolymer accompanying the helix-coil transition. Therefore, the red shift caused by ATP here may be due to an other conformational transition than that detected by the helical change. If it is the case, a bigger red shift of tyrosine band than that observed in this experiment must be caused by the transition as to overcome a blue shift accompanying the decrease in the helical content.

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