AN ATP-INDUCED CONFORMATIONAL CHANGE IN THE ACTO-H-MEROMYOSIN SYSTEM[1]

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Introduction: A recent theory of muscle contraction (Davies,1963) assumes that the myosin-bound ATP participates in calcium-chelated cross-bridges between the filaments of the myofibrils and further that during dephosphorylation by myosin ATPase (ATP phosphohydrolase EC 3.6.1.3) and rephosphorylation of this ATP, a polypeptide segment in the H-meromyosin region undergoes an α-helix to a mixture of random coil and extended β-form type of conformational change. Conformational changes in myofibrillar contractile protein systems have been observed by X-ray diffraction (Pautard,1958) and optical rotatory dispersion (Tonomura, Sekiya and Imamura, 1963, Kay and Brahms, 1963). The hypochromic effect on polypeptide chromophores due to the parallel alignment of the chromophores in the α-helix as observed in the far ultra-violet absorption band ($\sim$ 190 mμ) promises to be a sensitive method to follow changes in the helical content (Rosenheck and Doty,1961). We have now studied the effect of ATP, ADP, and AMP on the ultra-violet (195-186 mμ) absorption spectrum of acto-H-meromyosin at low ionic strength and the effect of calcium on the protein-nucleotide system.

Methods: F-actin, free from tropomyosin and ATP:AMP phosphotransferase (EC 2.7.4.3),was prepared from acetone-powders of rabbit skeletal muscle as described elsewhere (Iyengar and Weber,1964). Thrice-reprecipitated myosin

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from rabbit psoas muscle was digested with trypsin under the conditions described by Lowey and Holtzer (1959) and the H-meromyosin was further purified by ammonium sulfate fractionation (40-55% fraction) and was exhaustively dialyzed against 1 mM Na, K phosphate buffer (pH7.0). The acto-H-meromyosin complex was formed by mixing 1 part by weight of F-actin with 2.5 parts of H-meromyosin. The clear viscous solution was diluted with quartz-redistilled water just before use. Spectrophotometric measurements were made in a Cary 14 spectrophotometer using quartz cells provided with spacers to reduce the light path to 1.0 mm. 1.5 ml of the protein solution, appropriately diluted (to give $E_{190}^{0.1\ cm}$ of 1.2-1.5), was pipetted into the cuvettes. After recording the spectrum between 195-186 mμ, 0.015 ml of the relevant nucleotide solution was added with a microsyringe and the spectrum was rescanned after gentle mixing. Calcium, when present, was added either before or after the nucleotide. Turbidity measurements were done in a Brice-Phoenix light scattering photometer.

Results: F-actin, H-meromyosin and acto-H-meromyosin all have essentially similar spectra in the 195-186 mμ region with $\lambda_{max.}$ at 188, 191.5 and 189 mμ respectively. Table I summarizes the effects of addition of ATP, ADP and AMP (final concentration 10^{-5}M) on the absorbance of these proteins at 189 mμ. ATP had no effect on the spectrum of F-actin either in the presence or absence of calcium. Addition of ATP to H-meromyosin increased the absorbance at 189 mμ by 0.04 absorbance units. The presence of calcium enhanced this effect to 0.06 absorbance units. With acto-H-meromyosin (formed from 4 different preparations) the hyperchromic effect of ATP was higher (Δ absorbance of 0.09) than with H-meromyosin alone. While the magnitude of the effect of ATP on different protein preparations was somewhat variable, with a given preparation the ATP induced hyperchromicity was always greater with calcium (Δ absorbance 0.13) than without. The increase in absorbance produced by ATP was maximum at the peak (189 mμ). In many experiments (7 out of 12) the effect of calcium and ATP was rapidly re-

TABLE I

Hyperchromic effect of calcium and nucleotides on actin, H-meromyosin and acto-H-meromyosin

Protein	Nucleotide	Calcium	No. of Expts.	Δ Absorbance at 189 mμ*	
F-Actin	ATP	+	1	0.00	(0.00)
		0	3	0.00, 0.00, 0.00	(0.00)
H-meromyosin	ATP	+	2	0.03, 0.08	(0.06)
		0	4	0.02, 0.05, 0.03, 0.07	(0.04)
	ADP	+	1	0.00	(0.00)
		0	1	0.00	(0.00)
Acto-H-meromyosin	ATP	+	6	0.15, 0.17, 0.11, 0.06, 0.07, 0.20	(0.13)
		0	4	0.10, 0.10, 0.03, 0.12	(0.09)
	ADP†	+	3	0.08, 0.05, 0.10	(0.08)
		0	1	0.12	(0.12)
	AMP	+	1	0.00	(0.00)
		0	1	0.00	(0.00)

1.5 ml of acto-H-meromyosin (0.13 mg/ml of F-actin + 0.28 mg/ml H-meromyosin) were treated with 0.015 ml of various nucleotides (final concentration 10^{-5}M). Calcium, when present, was added as $CaCl_2$ (final concentration 1.0 to 3.0 x 10^{-3}M).

*Figures in parenthesis are averages.
†See Table 2 and text for discussion of this effect.

TABLE 2

Comparison of the hyperchromic effect of ATP and ADP at different wave lengths

Protein	Nucleotide	Increase in Absorbance		
		195 mμ	189 mμ	187 mμ
Acto-H-meromyosin	ATP	0.08	0.20	0.19
	ADP	0.060	0.060	0.062

1.5 ml of protein solution was treated with 0.015 ml of 10^{-3}M nucleotide. Calcium concentration was 10^{-3}M.

versed (in 2-10 minutes) and the spectrum reverted to that of acto-H-meromyosin. It was observed that preparations which showed rapid reversal when fresh, failed to reverse after 2 days at 4°C. ADP had no measurable effect on H-meromyosin. It is interesting that with acto-H-meromyosin ADP

seemed to have an effect similar to that of ATP though the magnitude of the change was smaller (Δ absorbance 0.08). However the change in absorbance produced by ADP in contrast to the effect of ATP, was of the same magnitude in all parts of the spectrum examined (Table 2). ADP apparently produces aggregation of the protein resulting in increased turbidity. This was further substantiated by measurement of turbidity. The scattered light at 90° (540 mμ) by acto-H-meromyosin went down on addition of ATP due to the dissociation of the acto-H-meromyosin complex, whereas the addition of ADP resulted in an increased scattering of light. The addition of AMP had no effect on acto-H-meromyosin.

Discussion: The hyperchromic effect observed in the 190 mμ region during the helix-coil transformation of synthetic polypeptides and proteins has been attributed to the disruption of the α-helix (Rosenheck and Doty,1961). In the present study ATP produced a hyperchromic effect on acto-H-meromyosin system. We interpret this result to mean that the binding of ATP to H-meromyosin in these experiments results in a decrease in the helical content. Since pure actin but not pure H-meromyosin was unaffected by ATP, this conformational change, we believe, occurs in the H-meromyosin molecule and that for maximum effect the presence of actin and calcium are required. The rapid reversal of the ATP effect observed in the presence of calcium probably follows from the activation of the H-meromyosin ATPase which then dephosphorylates the ATP.

Interestingly, Pautard (1958) found that when actomyosin was synaraesed at low ionic strength by ATP, the X-ray diffraction indicated disappearance of the α-structure and appearance of "cross-β-structure". However, optical rotatory dispersion studies (Tonomura et al., 1963) on actomyosin and acto-H-meromyosin done at high ionic strength (0.3 to 0.6 M KCl) indicated that ATP increased the helical content of the ATPase while pyrophosphate had the opposite effect. Unfortunately, our measurements could not be made under their conditions due to the high absorbance

of KCl in the 190 mμ region.

We conclude that ATP in the presence of actin and calcium disrupts the helical structure in some part of the H-meromyosin molecule and that when the ATP has been dephosphorylated by the calcium-activated ATPase, the protein returns to its original conformation.

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