determined (*7*), and the radioactivity in nuclear RNA was assayed (*7*). Within as short a time as 70 minutes after administration, 0.5 mg of aflatoxin B_1 markedly blocked cytidine incorporation into nuclear RNA and lowered the ratio of nuclear RNA/DNA (Table 1). Although the inhibition of cytidine-H^3 incorporation into nuclear RNA may result merely from inhibition of transport of RNA precursors to intracellular sites of RNA synthesis, the lowering of the ratios of nuclear RNA to DNA shows directly that the RNA content of the nucleus has diminished. The results suggest that a primary effect of aflatoxin B_1 is the blocking of RNA synthesis in the nucleus. Moreover, the alterations in RNA metabolism 17 hours after administration of aflatoxin were essentially the same as those at 70 minutes. The data are very similar to those (*7*) on the effects of actinomycin D on the metabolism of liver nuclear RNA in vivo.

In summary, aflatoxin B_1 itself has been shown to bind to DNA in vitro. The rapid and drastic alteration of nuclear RNA metabolism caused by aflatoxin B_1 in vivo strongly suggests that its ability to bind to DNA is a crucial aspect of its toxic and carcinogenic properties. The inhibitory effects of aflatoxin B_1 on mitotic division in cultured embryonic lung cells (*8*) and the ability of aflatoxin to induce chromosome aberrations in *Vicia faba* (*9*) are also consonant with such a mechanism. It would also appear that aflatoxin B_1 itself, rather than one of its metabolites, may be a proximate carcinogen. Further studies on the possible binding of aflatoxin B_1 to nucleic acids, proteins, and other cellular constituents in vivo will be required before the actual mechanism of the carcinogenesis by aflatoxins can be defined.

MICHAEL B. SPORN
C. WESLEY DINGMAN
HARRIETTE LONGACRE PHELPS
National Cancer Institute, Bethesda, Maryland 20014
GERALD N. WOGAN
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge

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Actin-Myosin Interaction: Inhibition of the Myosin Adenosine Triphosphatase by Actin

Abstract. *In the absence of magnesium ion, the addition of actin to myosin in a 1 : 4 ratio has a strong inhibitory effect on the adenosine triphosphatase activity, in contrast to the well-known activating effect of actin in the presence of magnesium ion. This finding suggests that both effects result from a conformational change in the active site of the myosin adenosine triphosphatase.*

The contraction of skeletal muscle depends upon a specific interaction between the contractile proteins, actin and myosin (*1*), and the energy for contraction is derived from the hydrolysis of adenosine triphosphate (ATP) (*2*). A striking feature of the actin-myosin interaction in vitro is the transformation of the magnesium-inhibited adenosine triphosphatase of myosin to the magnesium-activated actomyosin adenosine triphosphatase (*3*). This actin activation of the adenosine triphosphatase in the presence of magnesium is maximal when the actin-myosin ratio is about 1 to 4 by weight (*4*), which corresponds to the stoichiometric combining ratio of actin and myosin determined by physical methods (*5*).

It has been proposed that myosin can hydrolyze ATP either directly by simple hydrolysis of the Michaelis enzyme-substrate complex, or through the magnesium-dependent formation of a myosin-phosphate intermediate (*6*), and, furthermore, that actin activates the second pathway of ATP hydrolysis by facilitating the decomposition of this myosin-phosphate intermediate (*7*). If this mechanism alone accounts for the effect of actin on myosin, then in the absence of magnesium ion, when the myosin-phosphate complex cannot be formed, actin should have no effect on the enzymic activity of myosin. In this regard, it has been reported that actin inhibits the calcium-activated adenosine triphosphatase of myosin (*8*) and heavy meromyosin (*9*) in the absence of added magnesium, but this inhibition was evident only at very high ratios of actin to myosin.

We have investigated the effect of actin on the myosin adenosine triphosphatase at extremely low concentrations of both magnesium and calcium ions, and under these conditions we have found that actin has a very marked inhibitory effect. Furthermore, like the actin activation of the adenosine triphosphatase in the presence of magnesium, the inhibition is maximal at a 1 : 4 ratio of actin to myosin.

Myosin was prepared from rabbit muscle by the method of Szent-Györgyi (*1*), and actin was prepared according to Ulbrecht *et al.* (*10*), except that no magnesium was added during the preparation of either protein. The hydrolysis of ATP was recorded by an automatic *p*H-stat at *p*H 7. The ATP concentration at the beginning of the reaction was 2 m*M*, and the reaction was initiated by the addition of 2 mg of myosin per milliliter. The sample temperature was maintained at 25°C. Average reaction rates were determined for the hydrolysis of the first 25 percent of the ATP. To reduce the free magnesium concentration in the reaction mixtures, a chelating agent, 5 m*M* 1,2-diaminocyclohexanetetraacetic acid (DCTA) (*11*), was added. In addition, 1 m*M* ethyleneglycol-bis(aminoethyl-ether)tetraacetic acid (EGTA) (*11*) was included in all the samples to keep

Table 1. Effect of actin on the myosin adenosine triphosphatase at varying concentrations of magnesium ion. All samples contained, in addition to added $MgCl_2$, 5 m*M* DCTA, 1 m*M* EGTA, 2 m*M* ATP, 0.12*M* KCl, 2 mg of myosin per milliliter, and, in the actomyosin samples, 0.5 mg of actin per milliliter, in a total volume of 7.5 ml. Rates were determined by *p*H-stat at *p*H 7, and are expressed as the number of micromoles of ATP hydrolyzed per minute per milligram of myosin in the sample.

Added $MgCl_2$ (*M*)	Free Mg^{++} (*M*)*	Adenosine triphosphatase (μmole mg^{-1} min^{-1})		Inhibition by actin (%)
		Myosin	Actomyosin	
	2.8×10^{-9}	0.032	0.0097	70
2.5×10^{-5}	1.7×10^{-8}	.031	.015	52
5.0×10^{-5}	3.1×10^{-8}	.031	.018	42
2.5×10^{-3}	2.8×10^{-6}	.030	.25	(8.3-fold activated)
6.0×10^{-3}	5.0×10^{-5} (+1 m*M* MgATP)	.009	.035	(4-fold activated)

* Calculated by assuming that only ATP and DCTA bind Mg, that the effective dissociation constants at *p*H 7 are $2.8 \times 10^{-6}M$ for MgDCTA (*14*) and $5 \times 10^{-5}M$ for MgATP (*15*), and that the total contaminant Mg^{++} concentration is about 5 μM (*16*).

the free calcium ion concentration low regardless of variations in the concentrations of DCTA or $MgCl_2$.

Seventy percent inhibition of the myosin adenosine triphosphatase was observed at an actin-myosin ratio of 1 : 4 by weight (Table 1). This inhibitory effect of actin was evident only at a very low concentration of free magnesium ions; the addition of 25 or 50 μM $MgCl_2$ considerably increased the actomyosin adenosine triphosphatase activity even in the presence of 5 m*M* DCTA.

When 2.5 m*M* $MgCl_2$ was added, so that the free magnesium concentration was about 3 μM, the inhibitory effect of actin was abolished, and a considerable actin-activation of the myosin adenosine triphosphatase appeared, accompanied by superprecipitation of the actomyosin gel. When the molar concentration of magnesium exceeded that of DCTA, a typical "clearing" response (*4*) preceded the superprecipitation of the actomyosin. The observation that the effects of DCTA are completely reversed by the addition of excess $MgCl_2$ supports our assumption that the chelating agent acts by removing free magnesium and not by a direct interaction with the proteins. In addition, the observed adenosine triphosphatase activities of myosin (Table 1) show that no significant magnesium inhibition is observed until the amount of added $MgCl_2$ exceeds the DCTA present. This observation eliminates the possibility that the inhibitory effect of actin is due to additional contaminant magnesium introduced with the actin solution.

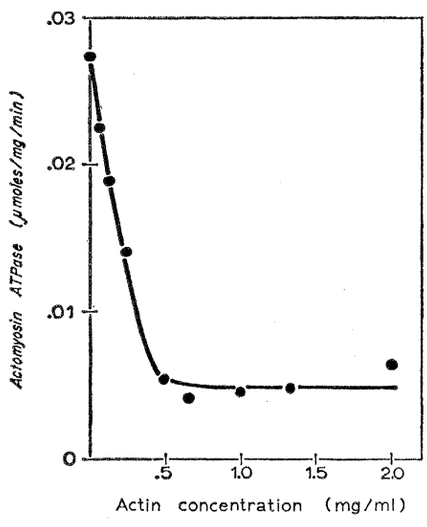

Fig. 1. Effect of varied actin concentration on the myosin adenosine triphosphatase at very low Mg^{++} concentration. All samples contained, in addition to the actin, 5 m*M* DCTA, 1 m*M* EGTA, 2 m*M* ATP, 0.12*M* KCl, and 2 mg of myosin per milliliter, in a total volume of 7.5 milliliters. Rates were determined by *p*H-stat at *p*H 7 and are expressed as the number of micromoles of ATP hydrolyzed per minute per milligram of myosin in the sample.

The rate of ATP hydrolysis decreases progressively with increasing actin concentration up to an actin-myosin ratio of about 1 : 4 (Fig. 1), after which further additions of actin are without effect. In view of the extreme sensitivity of the system to magnesium, the residual adenosine triphosphatase activity which remains even at actin-myosin ratios up to 1 : 1 can probably be ascribed to contaminant magnesium in the system. The fact that the inhibition curve in Fig. 1 is essentially a mirror image of the curve for actin-activation of the myosin adenosine triphosphatase in the presence of magnesium (*4*) indicates that the same complex formation between the two proteins produces either activation or inhibition, depending on the concentration of magnesium ion.

That the binding of actin can inhibit as well as activate the myosin adenosine triphosphatase suggests that, apart from any effect which actin may have in accelerating the breakdown of a myosin-phosphate intermediate, it also alters the hydrolytic site of the myosin adenosine triphosphatase. This alteration may occur through a conformational change induced by the binding of actin to a separate site on the myosin, since there is evidence that the actin-binding and enzymic sites of myosin can be selectively destroyed (*12*). A further indication of a conformational change is the slight decrease in helical content which accompanies the formation of the complex of actin with myosin or heavy meromyosin (*13*). In any case, the inhibitory effect of actin as well as its activating effect on the myosin adenosine triphosphatase should be considered in future proposals for the mechanism of the actin-myosin interaction.

S. Barron
E. Eisenberg
C. Moos

Department of Biophysics, State University of New York at Buffalo

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