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Affinity Labeling of Rabbit Muscle Myosin with a Cobalt(III)–Adenosine Triphosphate Complex†

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ABSTRACT: Several Co(III) and Cr(III) complexes have been tested for their ability to serve as affinity labels for the active site of myosin. The complex of Co(III)–ATP and phenanthroline [Co–(phen)–ATP] was studied in detail. Its binding to myosin is stable and affects the ATPase activity; it can, however, be displaced by the addition of thiol reagents, such as dithiothreitol, with consecutive recovery of the original activity. Full labeling yields two labels per myosin molecule, which is in accordance with the existence of two "heads" per myosin molecule, each probably carrying one active site. The kinetics of labeling suggests that binding abolishes the activity of a site and that when only one of the two heads is labeled, the activity of the unlabeled one is enhanced by a factor of 3.4–3.6 under the experimental conditions. Although the label does not seem to bind to –SH groups, it protects the essential –SH groups from modification by *N*-ethylmaleimide, probably by binding in the proximity of these groups. It appears that the label is located in the region of the active site thus serving as an affinity label of the active sites of myosin.

One of the key questions in muscle contraction is the mechanism of transduction of chemical into mechanical energy. The source of chemical energy for this process is ATP which is bound and hydrolyzed by myosin. It is thus of utmost importance to elucidate the details of the interaction between myosin and ATP, and both structural (Morita, 1967; Seidel and Gergely, 1971; Werber *et al.*, 1972; Viniegra and Morales, 1972; Murphy, 1973) and kinetic (Lymn and Taylor, 1971; Bagshaw *et al.*, 1972; Schliselfeld and Kaldor, 1973) work have been directed toward this goal.

In order to determine the structure of the active site of myosin it has previously been affinity labeled. This has been performed with reagents interacting specifically with thiol groups which are known to be necessary for activity (Murphy and Morales, 1970; Yount *et al.*, 1972, 1973). We have used a different approach for affinity labeling, based on the fact that in muscle the substrate is a complex of ATP with Mg^{2+}. Previous studies (Danchin, 1971, 1973; Danchin and Buc, 1973; Kowalsky, 1969) have indicated the potentialities of the use of Co(III) and Cr(III) derivatives for the affinity labeling of Mg^{2+}, or Mg^{2+}–substrate, binding sites in proteins and nucleic acids. We have therefore attempted to label the active site of myosin with various Co(III) and Cr(III) complexes. At least one of these, Co(III)–phenanthroline–ATP [Co–(phen)–ATP],[1] is shown in this study to behave as an affinity label of the active sites.

Experimental Section

Myosin and heavy meromyosin (HMM) were prepared as described by Azuma and Watanabe (1965) and by Lowey and Cohen (1962), respectively, from rabbit muscle. Both materials were stored at −18° in 50% glycerol, which was removed before use by dialysis against 5 mM phosphate buffer (pH 7.0), containing KCl (0.5 M in the case of myosin and 0.05 M in the case of HMM).

Synthesis of Co(III)–ATP Complexes. Co(III)–ATP, containing one molecule each of ATP and of Co^{3+} per molecule, was synthesized by electrophoresis and purified in a similar manner to the Co(III)–5′AMP complex described by Danchin and Buc (1973). All other complexes were synthesized as follows. A solution containing 11 mM ATP, 10 mM $CoCl_2$, and 10 mM complexing agent was slowly brought to pH 10 (in ice) with vigorous aeration which was stopped when no color increase was observed. The mixture was then purified by two precipitations in ethanol (2:1 v/v) at −20°. This removed most of unreacted reagents.

The complexing agents were: 1,10-*o*-phenanthroline (Merck), ethylenediamine (Koch-Light), and *N*,*N*′-dimethylethylenediamine (Merck), which will hereafter be denoted as phen, en-, and dimethyl-en, respectively.

Figure 1 shows the absorption spectrum of Co–(phen)–ATP

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[1] Abbreviations used are: phen, phenanthroline; HMM, heavy meromyosin; en, ethylenediamine; dimethyl-en, dimethylethylenediamine.

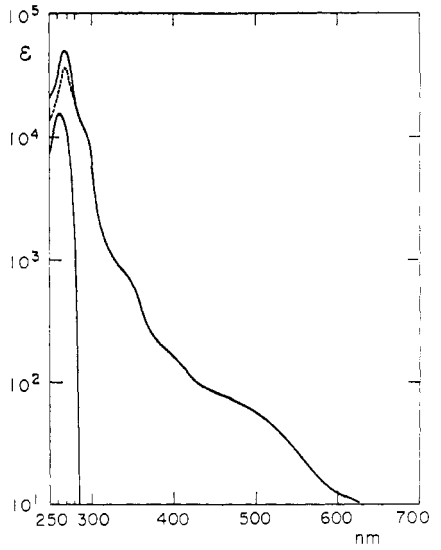

FIGURE 1: Absorption spectrum of the Co-(phen)-ATP complex. Measured after ethanol precipitation at pH 9.5. The extremum at 270 nm is mainly due to ATP and phenanthroline and the shoulder at 290 nm to phenanthroline absorption. The shoulders visible at 400 and 500 nm result from the d-d transition of Co(III). The dotted line is the difference between the actual spectrum (higher curve) and the ATP spectrum (lower curve).

thus prepared. At pH 7.0 the spectrum changed with time (half-lifetime: 20 hr at 0°) while at higher pH values it was more stable; the final spectrum corresponded to a mixture of Co^{2+}, phenanthroline, and ATP, which indicated that the complex has been decomposed into its original components. The ATP and the phenanthroline contents were determined from absorption measurements (at 260 nm and at 264 and 287 nm, respectively) and the cobalt content evaluated from the absorbance of the complex obtained in the presence of 2-mercaptoethanol (Danchin and Buc, 1973) at 480 nm (ϵ 9000), after separation of the reduced complex on a G-10 Sephadex column. The composition corresponded to a stoichiometry of 1:1:1 in the original complex. The elution profile of the complex on a Sephadex G-10 column at pH 10 yielded a molecular weight of 850 which corresponds to Co-(phen)-ATP. The spectrum in Figure 1 has several features suggesting the presence of the O_2^- ion as a ligand. Incidentally, pH values higher than 9.5, at which the complex is relatively stable, also favor the formation of many O_2^- complexes (Michelson, 1973). All this suggests the following reaction scheme for the formation and decomposition of the complex

$$Co^{2+} + \text{phenanthroline} + ATP + O_2 \rightleftharpoons Co^{3+}\text{-(phen)-ATP} + O_2^- \quad (1)$$

Labeling of Myosin and HMM. The labeling was carried out as follows. Myosin (3 mg/ml) was diluted in a 5 mM potassium orthophosphate buffer so that the final concentration of KCl was 120 mM; under such conditions myosin (but not HMM) is strongly aggregated. The label was added at a 100- to 300-fold excess and aliquots were removed at various time intervals for activity measurements. During the labeling process the solution was kept in ice.

The amount of Co-(phen)-ATP bound to myosin was determined from the absorbance at 480 nm of the Co(III)-dithiothreitol complex obtained by thiolysis of the labeled myosin; in this procedure labeled myosin was either extensively dialyzed or triply precipitated and centrifuged before dithiothreitol was added.

Results

Labeling Experiments and Stoichiometry of Co-(phen)-ATP Labeling of Myosin. Table I shows that the incubation of myosin for up to 2 days with some Co(III)-ATP complexes at 0° affects both its Ca^{2+}- and EDTA-ATPase activities. Once inactivation had occurred with these reagents it was irreversible, even after exhaustive dialysis (see also below). However, when myosin samples, labeled with the Co-(phen)-ATP complex and exhibiting less than 10% of the original ATPase activity, were treated with a thiol reagent (Danchin and Buc, 1973), such as dithiothreitol, about 90% of the original activity could be restored. The behavior of this complex was therefore examined in greater detail.

Figure 2 shows the inactivation curves of myosin treated with Co-(phen)-ATP at two different concentrations. The most remarkable feature is that both Ca^{2+}- and EDTA-ATPase activities are first *enhanced* and then decrease in a parallel manner. The activation phenomenon lasts for a longer period at the lower concentration of the labeling agent.

In order to determine the maximal number (n) of label molecules bound to a myosin molecule, the labeling of myosin (3 mg/ml) was performed at a complex concentration of 2 mM, *i.e.*, at a 330-fold molar excess of Co-(phen)-ATP. When both Ca^{2+}-ATPase and EDTA-ATPase activities were diminished to less than 2% of the original, excess reagent was removed and the concentration of Co(III) that remained bound to the enzyme was determined with dithiothreitol. The results obtained were as follows: $n = 2.1 \pm 0.3$ (removal of excess complex by repeated precipitation at low ionic strength) and $n = 2.0 \pm 0.2$ (removal of excess complex by dialysis for 48 hr). This is consistent with the observation that myosin has two nucleotide binding sites, each probably associated with one of myosin's two heads (Lowey and Luck, 1969).

The above results suggest the following reaction scheme

$$M + C \xrightarrow{k_1} MC \quad (2a)$$

$$MC + C \xrightarrow{k_2} MC_2 \quad (2b)$$

TABLE I: ATPase Activity of Myosin Incubated with Various Co(III)-ATP Complexes.

Complex	Complex Concn (M)	Time of Incubn[a] (hr)	% Activity Ca^{2+}-ATPase[b]	% Activity EDTA-ATPase[b]
Co-ATP	2×10^{-4}	22	105	
		51	138	
Co-(en)-ATP	1×10^{-3}	51	97	83
Co-(dimethyl-en)-ATP	1.5×10^{-3}	27	26	
		23	27	80
		51		65
Co-(phen)-ATP	4.8×10^{-4}	3	107	120
		8	64	45
		32	10	9.5

[a] Incubation conditions: myosin, 3 mg/ml in 125 mM KCl + 5 mM phosphate buffer, pH 7.0, kept in ice. [b] Assay conditions: aliquots of 0.05 ml (*i.e.*, 0.15 mg) withdrawn at various times and injected into 3 ml of assay solution containing 2.5 mM ATP (with 4 mM Ca^{2+}, 50 mM KCl for Ca^{2+}-ATPase, and with 1 mM EDTA, 500 mM KCl for EDTA-ATPase) at pH 7.5. Rates were determined in a pH-Stat, with 0.01 N NaOH as titrant.

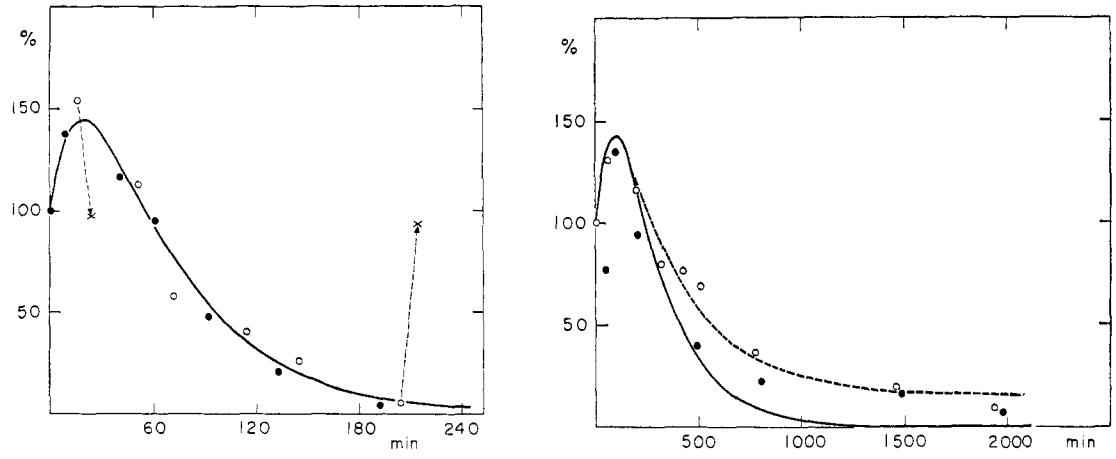

FIGURE 2: Irreversible inactivation of myosin activity in the presence of Co-(phen)-ATP. Filled and empty circles represent EDTA- and Ca^{2+}-ATPase activity, respectively. The solid lines were drawn on the basis of the scheme represented by eq 2 and the corresponding kinetic model (see Appendix) for the Ca^{2+}-ATPase activity. The fact that the points for EDTA-ATPase activity appear to be somewhat lower may be explained by assigning a smaller value for the stimulation factor. (A) The initial concentration of Co-(phen)-ATP was 2 mM. The crosses represent the activity after the addition of 10 mM dithiothreitol to labeled myosin. (B) The initial concentration of Co-(phen)-ATP was 0.48 mM. The dotted curve was drawn after introducing a correction for the spontaneous decomposition of the labeling reagent ($\tau_{1/2}$ = 20 hr at 0°).

where M denotes myosin and C the cobalt complex. We assume that, just as the ATPase activity drops down to zero when both myosin heads are labeled (see Figure 2), the activity of *each* of the heads is abolished when it binds one Co-(phen)-ATP molecule. The fact that the ATPase activity first *increases* with time would then mean that when the first site is blocked by the label, the activity of the second site is stimulated. If we denote the overall enhancement in the activity of such a myosin molecule by s then the activity of the free site will increase by a factor of $2s$. A mathematical analysis of this kinetic scheme (see Appendix) yields the following values: $k_1 = 0.622\ M^{-1}\ sec^{-1}$, $k_2 = 0.119\ M^{-1}\ sec^{-1}$, $s = 1.80$ and 1.69 for Ca^{2+}- and for EDTA-ATPase activities, respectively; the curves in Figure 2 are *theoretical*, using these values and after introducing a correction for the spontaneous decomposition of the labeling reagent.

The ATPase activity of heavy meromyosin (HMM) labeled with Co-(phen)-ATP is shown (Figure 3) to follow a monotonous course. The curve in Figure 3 was calculated assuming a single reaction with $k = 0.045\ M^{-1}\ sec^{-1}$, again taking into account the spontaneous decomposition of Co-(phen)-ATP.

Inhibition in the Presence of Substrate. The effect of various Co(III) complexes on the activity of myosin was also followed by adding aliquots of the complexes directly into the assay medium of myosin. In this case the inhibition by the complexes was performed in the presence of 2.5 mM ATP which is a saturating concentration of the substrate (Morita, 1967). The inhibition was immediate, *i.e.*, a few minutes after the addition of the complex at 25°. In most cases it has been observed that the inhibition in the medium for EDTA-ATPase activity was much less pronounced than in the case of the Ca^{2+}-ATPase medium. Therefore, only the results for the Ca^{2+}-ATPase medium are presented in Table II.

The case of Co-(phen)-ATP has been studied in more detail and inhibition measured in the presence of several ATP concentrations. Figure 4 shows that for concentrations of substrate higher than 0.4 mM (0.4–10 mM) the inhibition is competitive (with inhibitor concentration in the range 0.04–0.4 mM). However, as can be seen from the insert to Figure 4, the inhibitory pattern is *parabolic*. This suggests an interaction between inhibitory sites. At the lowest ATP concentrations the inhibitor's concentration becomes of the same or higher

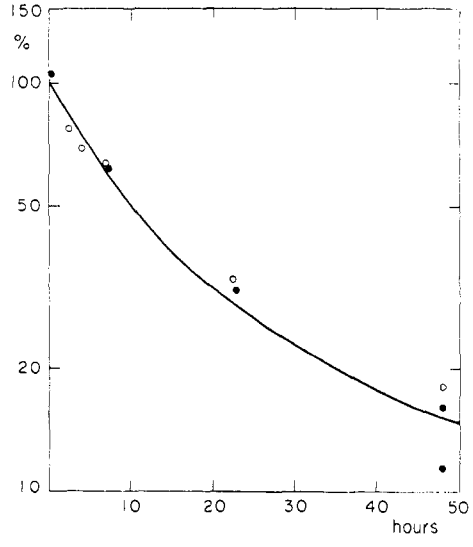

FIGURE 3: Irreversible inactivation of HMM in the presence of Co-(phen)-ATP. As in Figure 2. On drawing the (theoretical) curve, the spontaneous degradation of the labeling complex was taken into account.

TABLE II: Inhibition by Various Co(III)-ATP Complexes of ATPase Activity of Myosin in Its Assay Medium.

Complex	Complex Concn (μM)	% Act.[a]
Co-(phen)-ATP	132	56
Co-(dimethyl-en)-ATP	112	46[b]
Co-(en)-ATP	222	43[c]
Co-ATP	333	25
Co-adenosine	30	0

[a] Ca^{2+}-ATPase activity with 50 μg/ml (0.1 μM) of myosin. 2–3 min after the mixing of myosin and ATP, an aliquot of the complex was introduced into the assay medium, and the activity followed immediately. [b] Average of two experiments at pH 7.70. [c] At pH 8.60.

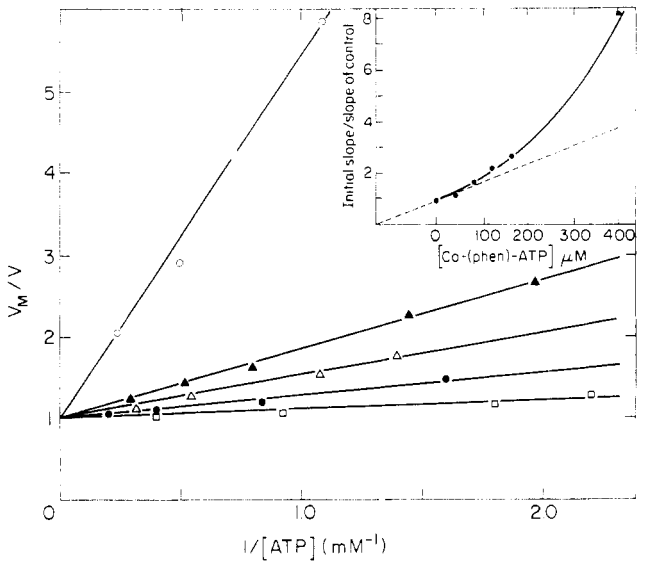

FIGURE 4: Inhibition of myosin Ca^{2+}-ATPase activity by Co-(phen)-ATP in the presence of ATP. The double-reciprocal plot indicates that inhibition is competitive; it is shown in the insert that this competitive inhibition exhibits a parabolic course: (□) 40 μM; (●) 80 μM; (△) 120 μM; (▲) 160 μM; (○) 400 μM of Co-(phen)-ATP.

order than that of ATP and the meaning of the assay becomes difficult to evaluate since Co-(phen)-ATP may itself be hydrolyzed to a Co-(phen)-ADP complex, thus contributing to the apparent activity.

Effect of Sulfhydryl Reagents on Co-(phen)-ATP-Labeled Myosin. In order to check whether the interaction of myosin with Co-(phen)-ATP involves -SH groups or protects them against modification, experiments with two thiol reagents, *p*-chloromercuribenzoate and *N*-ethylmaleimide were carried out. A *p*-chloromercuribenzoate titration (Riordan and Vallee, 1967) of the -SH groups in Co-(phen)-ATP-labeled myosin (which had been freed of excess reagent by dialysis) showed that it had lost four titrable -SH groups, when compared with control myosin.

The -SH groups of both labeled and control myosin were modified with *N*-ethylmaleimide, and the labeling released by dithiothreitol which does not affect the *N*-ethylmaleimide modification. The results of these experiments are shown in Table III. It is clear that the label protects some essential -SH groups against modification by *N*-ethylmaleimide.

Effect of Labeling Myosin with Co-(phen)-ATP on the Light-Chain Pattern. In an attempt to localize the site of labeling by Co-(phen)-ATP, polyacrylamide gel electrophoresis of the myosin in the presence of 6 M urea was performed. Under these conditions, dissociation of the light chains of myosin occurs (Gaffin and Watanabe, 1972) and it was assumed that if the label molecules bridge between heavy and light chains, then the pattern of light chains observed might differ from that of control myosin.

No significant difference was observed between the light-chain patterns of control myosin and that of Co-(phen)-ATP-labeled myosin. A light chain loaded with one or two molecules of label under the experimental conditions would have probably exhibited an electrophoretic mobility different from that of the free light chain (Gaffin and Oplatka, 1974).

Discussion

Among all the Co(III) complexes tested (Table I), Co-(phen)-ATP is the most suitable labeling reagent for myosin in spite of the fact that one has to use a relatively high concentration of complex (80-fold molar excess) in order to obtain a reasonable rate of labeling (as expressed by a loss of more than 90% of the ATPase activity in 32 hr at 0°). The facts which strongly suggest that Co-(phen)-ATP binds at the active site(s) of myosin are the following. (a) The activity inhibition in the presence of various ATP concentrations is clearly competitive (Figure 4). As can be seen from Table II, this property of myosin inhibition is shared by all Co(III)-ATP complexes investigated, even by those which are ineffective as irreversible labeling agents. However, the mechanism of inhibition in the case of complexes other than Co-(phen)-ATP has not been tested and, for instance, the fact that the inhibitory effect of Co-(dimethyl-en)-ATP is not reversed by dithiothreitol suggests that in some cases inhibition may not be competitive. (b) The stoichiometry of labeling (two sites per molecule) found in the case of Co-(phen)-ATP is consistent with the notion that it is the active sites which are labeled. Since complete labeling destroys activity, the label has to be strongly coupled with the active site; at the same time the fact that the labeling may be reversed by dithiothreitol suggests that the change induced by the reagent is not so drastic as to become irreversible. (c) The label protects thiol groups involved in activity since *N*-ethylmaleimide's irreversible association fails to destroy activity when Co-(phen)-ATP is present.

The most remarkable feature of the labeling process is that when one of the sites is labeled the ATPase activity of the second site is enhanced by a factor of 3.5, relative to the average ATPase activity per site. At the same time there is a 5-fold decrease in the labeling rate of the second site as compared with that of the first one. In order to *directly* prove that

TABLE III: Effect of *N*-Ethylmaleimide on the Ca-ATPase Activity (in % of Control) of Co-(phen)-ATP-Labeled Myosin.

Molar Ratio NEM:Myosin	Time of NEM[a] Modification	ATPase Activity: Control Myosin	Control Myosin + DTT[b] 20 min	Control Myosin + DTT[b] Overnight	Labeled Myosin	Labeled Myosin + DTT[b] 20 min	Labeled Myosin + DTT[b] Overnight
0		100	103		<2	98	
39	4 hrs	47	52		<2	94	
78	1 hr	31	32		<2	76	
400	45 min	27			<2		
400	80 min		32	21	<2	12	21

[a] NEM = *N*-ethylmaleimide. [b] DTT = dithiothreitol.

loss of activity was due to the binding of a second molecule of the complex and not to a rearrangement of already bound labels, a partially labeled myosin was freed of excess reagent by repetitive precipitations and centrifugations and kept in ice: there was almost no further decrease in activity with time.

The effect of *anticooperativity* seems to disappear during the labeling of HMM which is slower even in comparison with the labeling of the second site of myosin under the same conditions. The difference in behavior between HMM and myosin may be related to the fact that at the low ionic strength of the experiment myosin, contrary to HMM, aggregates into filaments. This might also account for the fact that the competitive inhibition by Co–(phen)–ATP is parabolic and not linear. The rodlike part of myosin, although very far away from the active sites, may thus serve to maintain some kind of interaction between sites. Experiments to verify this and other interpretations are now in progress.

The preliminary attempt to localize the site of association of Co–(phen)–ATP using the light-chain patterns obtained by gel electrophoresis of labeled and control myosins does not seem to indicate that the label binds to any of the light chains.

Up until now, –SH reagents have always been reported to cause a *decrease* in the EDTA–ATPase activity of myosin (Samaha *et al.*, 1970; Yount *et al.*, 1972). Our finding that the binding of the cobaltic ion to myosin initially causes an increase in this activity might indicate that –SH groups are not directly involved in the binding. Thiol complexes of Co(III) derivatives have a characteristic absorption band centered around $\lambda = 480$ nm and this is not observed with Co–(phen)–ATP-labeled myosin. However, considering the relatively low absorbance of Co(III)–cysteine complexes (Kothari and Busch, 1969), the spectral evidence does not rule out the possibility that a –SH group is involved. On the other hand, both the experiments with *N*-ethylmaleimide and the titration of –SH groups by *p*-chloromercuribenzoate suggest that some –SH groups are protected from modification by the labeling with Co–(phen)–ATP. At relatively low *N*-ethylmaleimide to myosin ratios, the potential activity is protected by the label (Table III). This protection, however, is abolished at higher ratios, where Co–(phen)–ATP-labeled myosin regains only a small fraction of its original activity after removing the labeling with dithiothreitol. Thus, although Co–(phen)–ATP does not seem to bind to –SH groups in myosin, it protects essential –SH groups from *N*-ethylmaleimide modification by binding to sites in the proximity of these groups. Thus, one can tentatively conclude that the label Co–(phen)–ATP is located at the site(s) where Mg–ATP binds. Experiments aimed to confirm this hypothesis and to isolate the peptides containing the essential –SH groups are now under way.

As has previously been stated, the Co(III) ion has already been shown (Danchin, 1971, 1973) to be a good analog of the Mg^{2+} ion, possibly because it has a very similar ionic radius. The addition of the phenanthroline ligand to the Co(III)–ATP complex markedly enhances its labeling ability to myosin. Mixed complexes of the type amine–metal ion–nucleotide are known (Huber and Sigel, 1973) to have a higher stability than that of the corresponding metal ion–nucleotide complexes, especially when the amine contains a π system. The enhanced affinity may thus be related to the increase in stability of the complex. Moreover, metal ion complexes containing aromatic nitrogens, such as phenanthroline, have been shown to possess special catalytic properties in various systems (Rund and Claus, 1964; Sigman *et al.*, 1972; Werber and Shalitin, 1973). Both the hydrophobic nature of the ligand and the possibility of delocalization of electrons into the π system of the amine may play a role in its remarkable properties. Experiments directed toward the characterization and elucidation of the structure of the labeling reagent Co–(phen)–ATP are now being carried out.

The labeling reagent, Co–(phen)–ATP is thus a promising tool for the elucidation of the structure of the active site of myosin. Since the substrate of myosin *in vivo* is in all probability Mg–ATP, this reagent could also possibly be an effective label for other Mg–ATPases, such as membrane ATPases (Skou, 1965).

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Appendix

Labeling of Interacting Myosin Heads. The most simple equations are eq 2a and 2b. For the sake of simplicity let us assume that the labeling process is a first-order reaction (actually the interactions are reversible associations which are followed by irreversible processes). Then

$$d[MC]/dt = k_1[M][C] \quad (1A)$$

$$d[MC_2]/dt = k_2[MC][C] \quad (2A)$$

Since the complex is in the large excess compared to total myosin, M_0

$$[C] = C_0 \simeq \text{const and } [M] + [MC] + [MC_2] = M_0 \quad (3A)$$

The ratio of the ATPase rate (V) to that of the control (V_m) is, at saturating ATP level

$$V/V_M = ([M] + s[MC])/M_0 \quad (4A)$$

Equations 1A to 3A yield

$$d^2[MC]/dt^2 + k_1[C]_0 \frac{d[MC]}{dt} + k_1k_2[C]_0^2[MC] = 0 \quad (5A)$$

which is easily solved, taking $[MC]_0 = 0$ and $d[MC]_0/dt = k_1[C]_0[M]_0$. With $\Delta \equiv (k_1[C]_0)^2[1 - (4k_2/k_1)]$ and

$$\lambda_1 \equiv \frac{k_1[C]_0 - \sqrt{\Delta}}{2}$$

$$\lambda_2 \equiv \frac{k_1[C]_0 + \sqrt{\Delta}}{2}$$

one obtains

$$[MC] = (k_1[C]_0[M]_0/\sqrt{\Delta})(e^{-\lambda_1 t} - e^{-\lambda_2 t}) \quad (6A)$$

Since $[MC_2]_0 = 0$, eq 6A and 2A yield

$$[MC_2] = [M]_0[1 - (\lambda_2/\sqrt{\Delta})e^{-\lambda_1 t} + (\lambda_1/\sqrt{\Delta})e^{-\lambda_2 t} \quad (7A)$$

Equations 3A to 7A give readily the time dependence of V

$$[S](t) \equiv V/V_M = \left[\frac{(s-1)k_1[C]_0 + \lambda_2}{\sqrt{\Delta}}\right]e^{-\lambda_1 t} - \left[\frac{(s-1)k_1[C]_0 + \lambda_1}{\sqrt{\Delta}}\right]e^{-\lambda_2 t} \quad (8A)$$

The properties of V/V_M are summarized as follows

t		0	t_m	$+\infty$
	$s > 1$	$(s-1)[C]_0k_1$	0	0
$\dot{S}$	$s = 1$	0		0
	$s < 1$	$(s-1)[C]_0k_1$		0
	$s > 1$	1 ⟶	$[S]_m$ ⟶	0
S	$s = 1$	1 ⟶		0
	$s < 1$	1 ⟶		0

with $t_m = \frac{1}{\sqrt{\Delta}} \log \left(\frac{\lambda_1\lambda_2 + \lambda_2(s-1)[C]_0k_1}{\lambda_1\lambda_2 + \lambda_1(s-1)[C]_0k_1} \right)$

$$S_m = \frac{1}{\lambda_1\lambda_2}[\lambda_1\lambda_2 + \lambda_1(s-1)[C]_0k_1]^{\lambda_2/\sqrt{\Delta}} [\lambda_1\lambda_2 + \lambda_1(s-1)[C]_0k_1]^{-\lambda_1/\sqrt{\Delta}}$$

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