Analysis of the Interaction of Rabbit Skeletal Muscle Adenylate Deaminase with Myosin Subfragments

A KINETICALLY REGULATED SYSTEM* (Received for publication, August 18, 1983)

Bruce A. Barshop and Carl Frieden
From the Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, Missouri 63110

The interaction of rabbit skeletal muscle adenylate deaminase with myosin fragments (heavy meromyosin and subfragment-2) has been studied by analytical centrifugation, gel chromatography, and stopped flow light scattering. Formation of the complex is highly cooperative with respect to addition of two molecules of adenylate deaminase/molecule of myosin fragment to form a ternary complex. Ternary complex formation is also highly pH-dependent with less complex formed at higher pH values, and the pH dependence is steeper with heavy meromyosin than with subfragment-2. At pH 6.5, the dissociation constant for the heavy meromyosin-deaminase complex is approximately 1.2 x 10^-15 M. Over the pH range 6.5-7.0, rate constants for the formation and dissociation of both the ternary and binary complexes of adenylate deaminase with heavy meromyosin have been determined. From analysis of the time course of stopped flow light scattering, the association steps are found to be extremely rapid, while the rate constant for dissociation of the first molecule of adenylate deaminase from the ternary complex is quite slow. This rate constant increases as the pH increased, but is sufficiently low that the interacting system does not equilibrate on the time scale of mass transport experiments (sedimentation velocity and gel chromatography), and thus displays apparent "slow" behavior.

The kinetic regulatory properties of adenylate deaminase are influenced by heavy meromyosin and subfragment-2, particularly with respect to inhibition by GTP. The association and dissociation of adenylate deaminase and myosin fragments and the resultant changes in kinetic properties of the adenylate deaminase can markedly alter the time course of the enzymatic reaction. The time scale over which this interaction is modulated by changes in pH may have significance in the metabolism of exercising muscle.

Adenylate deaminase (AMP aminohydrolase, EC 3.5.4.6) is abundant in vertebrate skeletal muscle and has long been recognized as the enzyme responsible for ammonia production in exercising muscle (1). The exact physiological role of the enzyme is not yet clearly understood, although suggestions relating to its role include the control of glycolysis either by activating phosphofructokinase and pyruvate kinase by the ammonia produced (as shown in yeast (2)) or by altering nucleotide levels (AMP, IMP, ATP) which, in turn, influence the behavior of important regulatory enzymes. It is of interest that the muscle isozyme appears to be lacking in some individuals who experience muscle cramping following exercise (3), although other isozymes of the enzyme may be present in sufficient amounts to prevent serious effects. Thus, the clinical picture of these patients appears to be quite variable (4).

Since the enzyme is ubiquitous in all higher organisms, it would certainly appear to function in an important role in metabolism. Particularly important, however, with respect to muscle metabolism is the observation that adenylate deaminase is a dynamic component of the thick filament of vertebrate skeletal muscle. The deaminase binds to myosin, HMM, and subfragment-2 (5), as well as to synthetic (6) and natural (7) thick filaments and has been found, by double immunofluorescence studies (8), to bind to the ends of the A band in isolated myofibrils and myotubes grown in culture. In the present paper, we will characterize the binding of adenylate deaminase to heavy meromyosin (and subfragment-2) and show that it is a highly cooperative process dependent on pH. We have determined the rate constants for the process and will show that these can be characterized as representing a "slow" protein:protein interaction. Thus, the results are of interest with respect to mass transport properties (e.g. sedimentation velocity, gel chromatography) since most treatments of those properties assume rapid equilibration.

In addition, as indicated above, adenylate deaminase is a highly regulated enzyme whose activity is influenced by purine nucleotide binding to both inhibitory and activating sites (9). While the activity of the enzyme in the absence of effectors is not affected by binding to myosin fragments, we have shown that the kinetic behavior with respect to these effectors (i.e. GTP, ADP) is influenced by the interaction of the deaminase with subfragment-2 (9). We will show similar behavior with heavy meromyosin and explore this relationship to show that a time-dependent response of activity to effector binding (nucleotide and myosin) may also be important in the regulatory behavior. The overall picture which emerges is one of a highly complex regulated enzyme system.

MATERIALS AND METHODS

Protein Preparation—Adenylate deaminase was prepared from the back and hind leg muscle of New Zealand White rabbits according to the protocol of Smiley et al. (10). The native enzyme molecular weight is about 300,000. Polyacrylamide gel electrophoresis in the presence of

* This work was supported by National Institutes of Health Grant AM-13332 and National Institutes of Health Medical Scientist Training Grant GM-07200. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: HMM, heavy meromyosin; S-2, subfragment-2; TES, [N-tris(hydroxymethyl)methyl]-2-amino-ethanesulfonic acid.
Interaction of Adenylate Deaminase with Myosin Subfragments

... of sodium dodecyl sulfate shows multiple bands when frozen muscle was used as specified in the original protocol (10), with two major bands of apparent Mr
depending on the conditions used. We obtained a more homogeneous preparation when a rabbit was killed each time that the enzyme is prepared and when protease inhibitors are included (0.5 mg/ml soybean trypsin inhibitor, 1 mM benzamidine, 1 mM phenylmethylsulfonfonyl fluoride). The adenylate deaminase preparations using fresh muscle generally show only a single band of apparent Mr
depending on the conditions used. We obtained a more homogeneous preparation when a rabbit was killed each time that the enzyme is prepared and when protease inhibitors are included (0.5 mg/ml soybean trypsin inhibitor, 1 mM benzamidine, 1 mM phenylmethylsulfonfonyl fluoride). The adenylate deaminase preparations using fresh muscle generally show only a single band of apparent Mr
depending on the conditions used. We obtained a more homogeneous preparation when a rabbit was killed each time that the enzyme is prepared and when protease inhibitors are included (0.5 mg/ml soybean trypsin inhibitor, 1 mM benzamidine, 1 mM phenylmethylsulfonfonyl fluoride). The adenylate deaminase preparations using fresh muscle generally show only a single band of apparent Mr

RESULTS

Sedimentation Studies—We have shown previously (5) that adenylate deaminase binds to heavy meromyosin and subfragment-2, but does not bind to subfragment-1 (which contains the ATPase activity of myosin). We had previously found that at pH 6.5 in 0.15 M KCl and 0.01 M imidazole buffer (20°C), the stoichiometry with which adenylate deaminase binds HMM is 2:1 (mol/mol). When a mixing ratio of 2:1 is used, sedimentation velocity patterns show (as previously reported (5)) a new peak with a sedimentation coefficient of 20 S. Fig. 1 shows that at any mixing ratio other than 2:1, a 20 S peak is observed as well as a slower peak which has the sedimentation coefficient of whichever species is present in excess of the 2:1 stoichiometry (s20,w for HMM = 7.1 S, for adenylate deaminase = 12.0 S). Analysis of the sedimentation velocity patterns obtained with, for example, excess HMM shows that the amount of material in the 20 S peak accounts for all of the deaminase plus the mass expected if this peak represents a 2:1 complex. The quantity of material which

Fig. 1. Stoichiometric appearance of reaction boundary in sedimentation velocity. Schlieren patterns are shown for HMM at 1.89 mg/ml (a), adenylate deaminase at 1.80 mg/ml (b), adenylate deaminase at 0.49 mg/ml plus HMM at 0.14 mg/ml (c), and adenylate deaminase at 0.49 mg/ml plus HMM at 0.54 mg/ml (d). Conditions are 0.15 M KCl with 0.01 M imidazole buffer (a and b) or 0.01 M TES buffer (c and d) adjusted to pH 6.5 at 20°C. Photographs are taken after 16 min at 40,000 rev/min, with the phase plate angle set to 55°.

...
sediments at 7.1 S accounts for the remainder of the HMM. These results indicate that little or no 1:1 complex is formed. Similar results are obtained when S-2 is substituted for HMM. It is of interest that the sedimentation coefficient for the deaminase:S-2 complex is approximately the same as that of the deaminase:HMM complex.

Sedimentation equilibrium experiments were performed to confirm that the molecular weight of the complex was that expected from a 2:1 stoichiometry. The results were analyzed using the omega function of the concentration distribution of an interacting system (18). The experimental results (not shown) indicate no significant heterogeneity and, even in the presence of excess HMM, no significant amount of complexed species of molecular weight below that expected for a 2:1 stoichiometry. However, further interpretations must be made with caution since there was an indication of material of higher molecular weight than expected, suggesting a small amount of a higher molecular weight aggregate. We believe, however, that the amount of higher aggregate is so small as not to influence any of the data analysis to be described below. In sedimentation equilibrium experiments on the individual proteins, the molecular weight of HMM was found to be 340,000 ± 5,000, while that for deaminase was found to be 300,000 ± 10,000 (data not shown).

Based on these observations and those presented below, we believe the reaction of adenylate deaminase and myosin subfragment to be

\[
M + A \rightarrow MA \\
MA + A \rightarrow MAA
\]

**MECHANISM I**

where \(M\) is the myosin subfragment and \(A\) is adenylate deaminase. Furthermore, the results imply a high degree of cooperativity with respect to the binding of the 2 mol of deaminase.

**Kinetic Studies of the Deaminase:HMM and Deaminase:S-2 Interaction**—In order to determine rate constants for the steps shown in Mechanism I, the kinetics of the association and dissociation processes (Mechanism I) were studied by light scattering changes using a stopped flow apparatus (as described under “Materials and Methods”). To correlate the observed scattering changes with molecular weight changes, samples of the complex (preincubated at 2:1 stoichiometry) were mixed with 0.01 M phosphate (all at pH 6.5). Since we have shown (5) that 0.01 M phosphate completely dissociates the complex, we could obtain the light scattering differences due to dissociation. An experiment of this type not only gives

![Fig. 2. Concentration dependence of light scattering changes.](image)

**Fig. 2. Concentration dependence of light scattering changes.** Adenylate deaminase and HMM preincubated at a stoichiometric ratio of 2:1 in 0.15 M KCl, 0.01 M imidazole, pH 6.5, 0.5 mM dithioerythritol were mixed with an equal volume of the same buffer containing 10 mM Pi. The overall change of light scattered at 90° is plotted as a function of final total protein concentration. Data are shown at a wavelength of 300 nm using a xenon arc lamp.

![Fig. 3. Light scattering changes accompanying association and dissociation under conditions of different pH.](image)

**Fig. 3. Light scattering changes accompanying association and dissociation under conditions of different pH.** Top shows light scattering data collected at pH 6.5 for the association of 1.25 \(\mu\)M adenylate deaminase (0.38 mg/ml) and 0.63 \(\mu\)M HMM (0.21 mg/ml) (a), the association of 2.5 \(\mu\)M adenylate deaminase (0.75 mg/ml) and 1.25 \(\mu\)M HMM (0.42 mg/ml) (b), and the two-fold dilution of 2.5 \(\mu\)M adenylate deaminase preincubated with 1.25 \(\mu\)M HMM (c). Middle shows data collected at pH 6.7 for the association of 0.9 \(\mu\)M adenylate deaminase and 0.45 \(\mu\)M HMM (a), the association of 1.8 \(\mu\)M adenylate deaminase and 0.9 \(\mu\)M HMM (b), and the two-fold dilution of 1.8 \(\mu\)M adenylate deaminase and 0.9 \(\mu\)M HMM (c). Lower shows data collected at pH 7.0, with other conditions as in the middle. The smooth curves superimposed over each data record are simulated light scattering changes, using Mechanism I and the rate constants shown in Table I. Molecular weight values used were 300,000 for adenylate deaminase and 340,000 for HMM. Since the syringes of the stopped flow apparatus are of equal volume, the initial concentrations prior to mixing were 2-fold larger.
the light scattering change for complete dissociation but also shows that between 300 and 500 nm the scattering change is proportional to $\lambda^{-4}$, suggesting that the change is, in fact, due to light scattering. It can also be shown that the light scattering change at 300 nm due to dissociation by phosphate is directly proportional to the total protein concentration (Fig. 2).

The top of Fig. 3 shows the light scattering changes associated with mixing adenylate deaminase and HMM at two different deaminase:HMM ratios. Also shown is the observed dissociation of the complex by a 2-fold dilution. The middle and bottom of Fig. 3 show similar data at pH 6.7 and 7.0, respectively. The solid lines given in the figure are those obtained using the mechanism given in Mechanism I with the stimulation method described under “Materials and Methods.” The rate constants obtained from the fit of the real data are shown in Table I. Values of $k_{1}$ and $k_{2}$ are uniquely determined from experiments of the type shown in Fig. 3. The values of $k_{1}$ and $k_{2}$ shown are consistent with the values of $k_{1}$ and $k_{2}$ used and give the best fit to the experimental data. For example, in order to fit the data, $k_{2}$ can not be less than $k_{1}$, and therefore was chosen as equal to $k_{1}$. $k_{1}$ can then be determined reasonably well from the data. These values will be discussed below (see “Discussion”).

It can also be seen in Fig. 3 that the extent of the light scattering change decreases at the higher pH values. This observation suggests that the degree of interaction between adenylate deaminase and HMM is pH-dependent. In order to study this dependence, we performed sedimentation velocity experiments at a deaminase:HMM ratio of 2:1 in 0.15 M KCl, 0.01 M TES buffer (20°C) from pH 6.8 to 7.9. At each pH, a duplicate sample was run in the presence of 0.01 M phosphate to show the pattern of the completely dissociated material. Fig. 4 shows the patterns observed at four pH values in the presence and absence of phosphate. It can be seen that at the intermediate pH conditions, three peaks are obtained. Fig. 5 shows the pH dependence of the peak areas. The curves drawn through those data with HMM and using Equation 1 show the transitions expected for an Adair-type titration with an apparent pK for the dissociation of around 7.55 and a value of $n = 2$. The results of similar experiments using S-2 over the pH range of 6.5–7.3 are also shown in Fig. 5. The curves drawn through the data with S-2 are those expected for a transition with a pK of 7.0 and a value of $n = 1$ (see Equation 1). These results not only indicate that HMM binds adenylate deaminase more tightly than does S-2 over this range of pH but that the dissociation of the deaminase:HMM complex involves two protons, while that for the deaminase:S-2 complex involves only a single proton. At each pH value shown in Fig. 5, the loading molar ratio of adenylate deaminase to myosin subfragment was 2:1 and at each pH, regardless of how much complex is formed, the areas of the slow sedimenting peaks indicate a 2:1 molar ratio of free adenylate deaminase to myosin subfragment. However, at all pH values where the complex is formed, there are 2 mol of deaminase bound per mol of either HMM or S-2.

The results of the sedimentation and light scattering experiments suggest that the binding of two deaminase molecules/HMM molecule is a highly cooperative process at pH 6.5, 6.7, or 7.0. In this regard, it was of interest that the sedimentation velocity experiments showed three clearly resolved peaks in the sedimentation pattern. Such behavior is not expected for a system of the type shown in Mechanism I.
or no effect on the adenylate deaminase activity, similar to
the observation for S-2 (line a in Fig. 7 or 8, see legend).
However, HMM protects against GTP inhibition of deami-
nase. Fig. 7 shows that when enzyme is preincubated with
HMM and then mixed with AMP and 10 μM GTP, the
appearance of GTP inhibition is delayed (compare line c
which is without HMM to line b in which the enzyme is
preincubated with HMM). Fig. 8 shows that when deaminase alone is
preincubated with 1 μM GTP and then mixed with AMP and
HMM, activation of the enzyme by AMP is accelerated (com-
pare line b (with HMM) to line c (without HMM)). In these
experiments, the HMM is not present with GTP long enough
to cause any significant hydrolysis to GDP.

Complete analysis of these and similar kinetic data is
difficult, to say the least. Such an analysis would necessarily
include 1) the hysteretic GTP inhibition and AMP activation,
as well as 2) the GTP-dependent dissociation of the complex

if the protein:protein interactions reflect rapid equilibrium
processes even when there is cooperative binding. In
order to examine the question of rapid or slow equilibration of the
proteins, zonal gel filtration experiments were performed. The
result of one such experiment is shown in Fig. 6. In this case,
deaminase and HMM were mixed at pH 6.8 and passed as a
small zone through a column of Sephacryl S-300. It can be
seen that there is a bimodal elution pattern of deaminase deaminase
activity with one peak appearing at the same elution volume as
deaminase deaminase alone but another eluting in the void
volume of the column. This excluded deaminase deaminase
activity corresponds with the HMM ATPase activity. The
fact that the proteins remained associated to a considerable
extent over the time scale of the gel filtration experiment
(about 2 h) provides strong evidence that at pH 6.8 the rate
of dissociation of the complex is slow in relation to the time
of re-equilibration at each chromatographic plate. In an
experiment in which the complex formed at pH 6.5 was
chromatographed at pH 7.2, the deaminase activity eluted in
a single peak at the volume expected for deaminase alone (data
not shown). The question of rapid and slow dissociation of the
2:1 protein complex will be discussed below (see "Discussion").

Kinetic Studies Relevant to the Deaminase Activity in the
Adenylate Deaminase:HMM Complex—We have previously
discussed the regulatory kinetic properties of adenylate de-
aminase (9). Briefly, the enzyme contains an active site, an
inhibitory site (which primarily binds purine triphosphates),
and an activating site (for mono-, di-, and triphosphates). The
specificities of these sites overlap and the regulatory
kinetic responses are therefore quite complex. In addition, the
enzyme shows hysteretic behavior with respect to inhibition
and activation (9).

We have also shown previously that S-2 affects the kinetic
behavior of the deaminase only with respect to the regulatory
behavior. Thus, GTP inhibition was lost in the presence of
excess S-2 (9). Since deaminase is bound more tightly to
HMM than to S-2, it was of interest to re-examine this
question using HMM. Figs. 7 and 8 show some of the observed
results. HMM, in the absence of nucleotide effectors, has little

2 D. J. Cox, personal communication.
The adenylyl deaminase:myosin subfragment system is unique among the systems of interacting proteins whose mass transport properties have been studied in detail. This system is notable in that it displays apparent cooperativity and an apparent slow time scale of the dissociation process. The values for the rate constants determined by stopped flow light scattering and shown in Table I are of considerable interest. They show 1) that the interaction is cooperative for the binding of 2 mol of deaminase/mol of HMM, 2) that the association rate constants, and are of the magnitude expected for diffusion control, and 3) that the rate-limiting step in dissociation of the complex is the dissociation of the first deaminase molecule from the ternary complex. Furthermore, the value of this rate constant is relevant to the question of the observed schlieren patterns as discussed below.

**Schlieren Patterns of Interacting Systems: Relation to Slowness of Interaction**—The shapes of concentration boundaries in mass transport experiments of interacting systems have been used to distinguish reaction mechanisms. Asymptotic expressions to describe the concentration boundaries can be derived when diffusion terms are ignored and when the interaction is assumed to be in rapid equilibrium (19). Complete description of the sedimentation patterns in interacting systems, including kinetically controlled reactions and diffusion, is only possible through computer simulation (20). Simulation studies, including the effects of diffusion, have been performed for a system such as outlined in Mechanism I, assuming rapid equilibration (21). Simulations predict a maximum of two peaks in the schlieren pattern. The feature of the deaminase:myosin system which is most obviously inconsistent with the predictions for a rapidly equilibrating interaction (19, 21) is the presence of three peaks, rather than two. A likely explanation is that the rate of equilibration between the complex and its components is slow. Certainly, the bimodal elution of adenylyl deaminase activity during zonal gel chromatography at pH 6.8 (Fig. 6) provides strong evidence that the dissociation may be considered to be slow. It is difficult to state the point at which an interaction would be expected to give rise to slow behavior in mass transport. Previous studies (22) on the effects of kinetically controlled reactions on the sedimentation patterns of interacting systems have been limited to cases of homogeneous association. It was the conclusion of those computer simulation studies (22) that for a ligand-mediated dimerization reaction, distinct differences from rapid equilibrium are observed only when half-times for dissociation exceed 60 s (for a sedimentation time of 1500 s). The rate constants determined from light scattering for the deaminase:HMM interaction (Table I) predict half-times on that order under conditions of lower pH (20-30 s at pH 6.5), while at higher pH values the dissociation rates may be more than 1 order of magnitude greater (half-time of 1.8 s at pH 7.0). Even so, the sedimentation pattern still shows three clearly resolved peaks. Preliminary simulation studies performed recently (23) can generate trimodal patterns using the reaction mechanism of Mechanism I with the rate constants of Table I for pH 6.5.-7.

Further studies may help to clarify what exactly is the experimental parameter in relation to which an interaction may be said to be slow. Certainly the critical parameter is not simply the duration of the experiment, for the duration of the zonal chromatography experiment in Fig. 6 is on the order of hours. More likely, the critical parameter relates to the rate with which the complex is resolved from its components, and this is likely to be a complex function owing to the cooperation between transport of the components and the rate of formation of the complex.

**Cooperativity of Interaction**—The curves in Fig. 5 illustrate the two types of cooperativity may be involved in the interaction between adenylyl deaminase and myosin. First, the interaction appears to be cooperative for the addition of the second molecule of adenylyl deaminase to either HMM or S-2. When adenylyl deaminase and HMM are combined at a 2:1 stoichiometric ratio in sedimentation velocity experiments, the proportions of the slow peaks remain approximately constant at 2:1, independent of the extent of association. When the proteins at pH 6.5 are combined at any other ratio, sedimentation velocity shows a fast boundary (20 S) and one slow boundary (with the sedimentation coefficient of whichever protein is present in excess of a 2:1 deaminase:HMM ratio). It is extremely unlikely that a 1:1 complex would sediment at the same rate as a 2:1 complex, and thus the fact that the sedimentation coefficient of the fast peak remains constant even when the proteins are mixed at a deaminase:HMM ratio of 1:1 suggests that the interaction is cooperative for the formation of the 2:1 complex. Furthermore, there is no evidence of an appreciable amount of a 1:1 complex in sedimentation equilibrium experiments, even in the presence of excess HMM. The maximal binding ratio determined by co-precipitation (5) is 2 mol of adenylyl deaminase/mol of myosin. It is, however, uncertain that this cooperative feature is manifest in vivo, since the observed maximal binding ratio has been observed to be 1 mol of adenylyl deaminase/2 mol of myosin in native thick filaments (7) and this maximum is probably imposed by steric limitations (6). However, the observed cooperativity in vitro is interesting from the point of view of interchain interaction during conformational changes of the S-2 region (24) and the question of head-head interaction in myosin (23).

The dissociation curves in Fig. 5 also illustrate that with HMM the pH transition appears to be cooperative, involving two protons, whereas with S-2 the process more closely resembles a single ionization. We have no information to indicate which ionizable groups are involved, nor even on which protein they reside. The overall titration may involve groups on the myosin subfragment, deaminase, or both proteins. However, at least part of the pH dependence must be inherent in the myosin subfragment, since clear differences are observed between the pH dependence of deaminase binding to HMM and to S-2. The difference in pK between the HMM and S-2 titrations may relate to the absence of residues from the smaller S-2 fragment and/or to the presence of internal cleavages resulting from the use of papain.

**Regulatory Effects**—Several ligands have the effect of dissociating the deaminase:HMM complex. These effects are most likely mediated by their binding to adenylyl deaminase, since the most effective dissociating ligands are inhibitors of adenylyl deaminase. Phosphate, which inhibits adenylyl deaminase with an inhibition constant in the range of 1 mM (25), dissociates the deaminase:HMM complex with a first order rate constant of about 50 s⁻¹ at concentrations of 5-10 mM (data not shown). GTP, which inhibits adenylyl deaminase at concentrations below 10 μM, also dissociates the complex at low concentrations. It is of interest that the inhibitory effects of GTP are reversed when GTP is chelated with Mg²⁺ (26). We have also observed that the dissociating effect of the GTP analog, guanyl-5'-ylididodiphosphate, is at
least partly reversed by Mg$^{2+}$ (data not shown).

As discussed previously (9), certain nucleotides (especially ADP) have a slight activating effect on adenylate deaminase. However, other nucleotides (such as GTP) show an inhibitory effect followed by activation as the nucleotide concentration is increased. The effect of a nucleotide such as ADP is more

following the pH changes. The fraction of adenylate deaminase bound to thick filaments has been observed to increase after stimulation of rat muscles (28). What physiological role this may play is unclear at present.

**REFERENCES**

1. Lowenstein, J. M. (1972) *Physiol. Rev.* 52, 382-414
2. Yoshino, M., and Murakami, K. (1982) *J. Biol. Chem.* 257, 2922-2928
3. Fishbein, W. N., Armbrustmacher, V. W., and Griffin, J. L. (1978) *Science* (Wash. D. C.) 200, 545-548
4. Schumate, J. B., Katnik, R., Ruiz, M., Kaiser, F., Frieden, C., Brooke, M. H., and Carroll, J. E. (1979) *Muscle Nerve* 2, 215-216
5. Ashby, B., and Frieden, C. (1977) *J. Biol. Chem.* 252, 1869-1872
6. Koretz, J. F., and Frieden, C. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7186-7188
7. Koretz, J. F. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6205-6209
8. Ashby, B., Frieden, C., and Bischoff, R. (1979) *J. Cell Biol.* 81, 361-373
9. Ashby, B., and Frieden, C. (1978) *J. Biol. Chem.* 253, 8728-8735
10. Smiley, K. L., Jr., Berry, A. J., and Suelter, C. N. (1967) *J. Biol. Chem.* 242, 2502-2506
11. Ranieri-Raggi, M., and Raggi, A. (1980) *Biochem. J.* 189, 367-368
12. Holtzer, A., and Lowey, S. (1959) *J. Am. Chem. Soc.* 81, 1370-1377
13. Lowey, S., Slayter, H. S., Weeds, A. G., and Baker, H. (1969) *J. Mol. Biol.* 42, 1-29
14. Lowey, S., and Cohen, C. (1962) *J. Mol. Biol.* 4, 293-308
15. Wolfenden, R., Tomozawa, Y., and Bannman, B. (1968) *Biochemistry* 7, 3965-3970
16. Parrish, R. G., and Mommaerts, W. F. H. M. (1954) *J. Biol. Chem.* 209, 901-913
17. Barshop, B. A., Wrenn, R. F., and Frieden, C. (1983) *Anal. Biochem.* 130, 134-145
18. Jeffreys, P. D. (1981) in *Protein-Protein Interactions* (Frieden, C., and Nichol, L. W., eds) pp. 213-256, Wiley, New York
19. Gilbert, G. A., and Jenkins, R. C. L. (1980) *Proc. R. Soc. Lond. Ser. A* 295, 420-457
20. Cox, D. J., and Dale, R. S. (1981) in *Protein-Protein Interactions* (Frieden, C., and Nichol, L. W., eds) pp. 173-211, Wiley, New York
21. Davis, L. C., and Chen, M. S. (1979) *Arch. Biochem. Biophys.* 194, 37-48
22. Cann, J. R., and Kegles, G. (1974) *Biochemistry* 13, 1668-1874
23. Reisler, E. (1980) *J. Mol. Biol.* 138, 93-107
24. Sutoh, Ka., Sutoh, Ke., Karr, T., and Harrington, W. F. (1978) *J. Mol. Biol.* 126, 1-22
25. Lee, Y.-P., and Wang, M.-H. (1968) *J. Biol. Chem.* 243, 2260-2265
26. Wheeler, T. J., and Lowenstein, J. M. (1979) *J. Biol. Chem.* 254, 8994-8999
27. Dawson, M. J., Gadian, D. G., and Wilkie, D. R. (1978) *Nature (Lond.)* 274, 861-866
28. Shiraki, H., Miyamoto, S., Matsuda, Y., Momose, E., and Nakagawa, H. (1981) *Biochem. Biophys. Res. Commun.* 100, 1099-1103
Analysis of the interaction of rabbit skeletal muscle adenylate deaminase with myosin subfragments. A kinetically regulated system.
B A Barshop and C Frieden

*J. Biol. Chem.* 1984, 259:60-66.

Access the most updated version of this article at [http://www.jbc.org/content/259/1/60](http://www.jbc.org/content/259/1/60)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/259/1/60.full.html#ref-list-1](http://www.jbc.org/content/259/1/60.full.html#ref-list-1)