Steady-State Kinetics of Smooth Muscle Myosin†

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ABSTRACT: The kinetic properties of purified smooth muscle myosin, free of actin, have been examined. Analysis of the steady-state kinetic data revealed an intermediary plateau region on the substrate saturation curves. In addition, these data, when analyzed by Hill and Lineweaver and Burk plots, indicate both positive and negative cooperativity, suggesting at least four substrate binding sites. The plateau region was abolished when the kinetic measurements were made at pH 5.5 and 9.0. Both positive and negative cooperative effects were absent at pH 9.0 and hyperbolic kinetics was observed. In contrast, at pH 5.5, although the plateau region was abolished, the enzyme exhibited positive cooperativity of substrate binding. When either heated or urea treated enzyme was used for kinetic measurements: (i) the plateau region shifted toward higher substrate concentration range; (ii) the cooperativity of binding sites was lost at low substrate concentrations but was instead seen at higher concentrations; and (iii) the V_{max} was doubled. These data have been interpreted as due to ligand-induced conformational changes in the enzyme according to J. Teipel and D. E. Koshland, Jr. (1969).

The myosin-catalyzed hydrolysis of ATP^{4-} is apparently the source of energy for all types of muscle contraction (Huxley, 1973) but the mechanism of ATP hydrolysis has been studied in detail only with myosin from skeletal muscle (Trentham et al., 1976). Even with skeletal muscle myosin, the exact nature and total number of ATP binding sites remain a subject of debate (Wagner and Yount, 1975; Taylor and Sleep, 1976; Eccleston et al., 1976), and less is known about the nature of interaction of ATP with myosin from smooth and nonmuscle sources. Knowledge of these interactions is essential to understand fundamental mechanisms of contraction and motility.

In studies of the nature of the interaction of ATP with myosin from other than skeletal muscle, we observed the cooperative binding of ATP with platelet myosin and we reported that one of the binding sites of platelet myosin may be different from the catalytic site (Malik et al., 1973; Malik and Stracher, 1977). Since the platelet contractile system is similar to smooth muscle (Malik et al., 1974; Cohen et al., 1976), it was of interest to see if smooth muscle myosin also exhibits cooperativity of binding sites. To investigate this and to learn more about the mode of interaction of ATP with smooth muscle myosin, I investigated the steady-state kinetics of chicken gizzard smooth muscle myosin. The data indicate a complex kinetic behavior of gizzard myosin with an intermediary plateau region on the substrate saturation curves. In addition, mixed positive and negative cooperativity of ATP binding has been observed.

Experimental Procedure

Methods

Myosin was purified from chicken gizzards according to a modification of the method outlined by Barany et al. (1966). All purification procedures were carried out at 0–4 °C except step 1, which was done at room temperature (23 °C).

Step 1: Homogenate. Fresh chicken gizzards from a local meat market were cleaned of fat and tough inner lining and minced in a meat grinder. Minced muscle (100–200 g) was washed with stirring at room temperature for 5–10 min. Seven to eight such washings were needed to completely remove the blood from the muscle. The washed muscle containing water (total volume approximately 1 L) was blended for 15–30 s. The homogenate was centrifuged at 9000 rpm for 15 min using a Sorvall RC2-B centrifuge.

Step 2: High Salt Extract. The residue obtained at step 1 was extracted with a solution containing 0.6 M KI, 20 mM phosphate buffer, pH 7.0, and 1 mM dithiothreitol (final concentration). Extraction was performed with stirring for 10 min. The homogenate was centrifuged at 9500 rpm for 10 min using a Sorvall RC2-B centrifuge. The supernate was filtered through four layers of cheesecloth and is referred to as the high salt extract.

Step 3: Precipitation of Actomyosin. The high salt extract from step 2 was dialyzed overnight against deionized water containing 1 mM dithiothreitol to a final salt concentration of 35 mM. The precipitated actomyosin was removed by centrifugation at 11 000 rpm for 20 min and was dissolved in KCl to a final concentration of 0.6 M. This solution was centrifuged at 11 000 rpm for 10 min for clarification. Any pellet was discarded. The crude actomyosin solution was diluted with deionized water to a final KCl concentration of 0.3 M and was centrifuged at 11 000 rpm for 15 min. A large pellet and a clear supernatant solution were obtained. The pellet was found by sodium dodecyl sulfate gel electrophoresis to contain actomyosin and other proteins and was discarded.

Step 4: Precipitation of Myosin. The clear supernatant solution from step 3 was diluted to a final KCl concentration of 35 mM, dithiothreitol was added to 0.5 mM and the solution was allowed to stand for 1–2 h at 4 °C. It was then centrifuged at 9500 rpm for 15 min. The pellet was dissolved in KCl to a final concentration of 0.6 M, the solution was clarified at 12 000 rpm for 15 min, and any pellet was discarded.

Step 5: Ribonuclease Treatment. The protein concentration was determined in the supernatant solution obtained at step 4, the solution was adjusted to pH 6.8, and was then diluted to a final KCl concentration of 37.5 mM. Ribonuclease was

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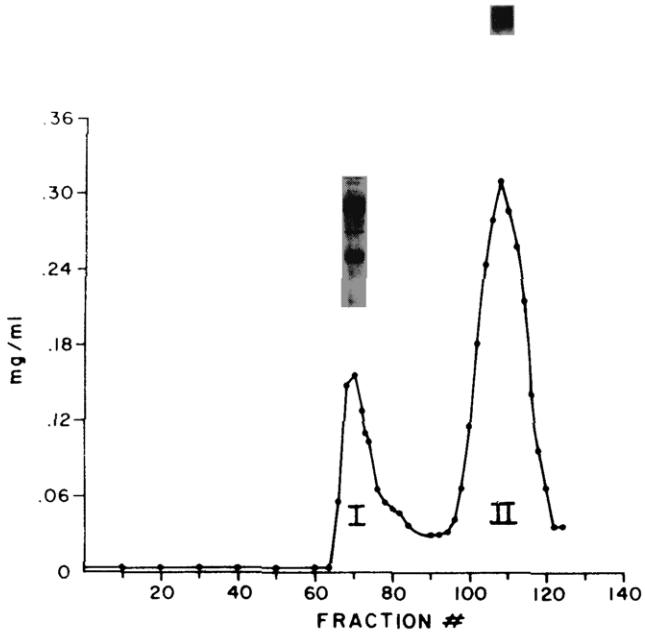

FIGURE 1: Chromatography of gizzard myosin. A 10-mL sample containing 30 mg of protein was chromatographed on a 2.5 × 90 cm column of Sepharose 4B. Fractions were pooled under each peak and analyzed by sodium dodecyl sulfate gel electrophoresis as indicated. For other conditions, see Experimental Procedure.

added to this suspension at a 250:1 ratio (myosin:ribonuclease) by weight and dithiothreitol was added to 0.5 mM. The mixture was allowed to stir overnight in the cold room. The myosin was collected by centrifugation at 9000 rpm for 15 min and was dissolved in 0.6 M KCl, 50 mM Tris-HCl, pH 7.5. The resulting solution was centrifuged for clarification and any pellet was discarded.

Step 6: First Ammonium Sulfate Fractionations. Solid $(NH_4)_2SO_4$ was added with gentle stirring to the supernatant solution from step 5 to a final concentration of 35%. The suspension was kept at 4 °C for 30 min and was then centrifuged at 11 500 rpm for 20 min. The pellet was discarded. To the supernatant solution, solid $(NH4)_2SO_4$ was added with gentle stirring to a final concentration of 55%. The suspension was centrifuged at 12 000 rpm for 15 min, and the pellet was dissolved in 0.6 M KCl, 5 mM Tris[1]-HCl, pH 7.4, and was dialyzed against a large excess of the same solution overnight. This will be referred to as the myosin solution.

Step 7: Sepharose 4B Chromatography. A Sepharose 4B column (2.5 × 90 cm) was equilibrated with 0.6 M KCl, 5 mM Tris-HCl, pH 7.5, 1 mM Ca-ATP^{2-} and 1 mM dithiothreitol. (Hereafter this solution will be referred to as buffer A.) Buffer A was also added to the myosin solution obtained in step 6 and this solution was then applied to the column. Finally the column was eluted with buffer A at a flow rate of 12–14 mL/h. As shown in Figure 1, two major peaks were eluted from the column, with myosin mainly in peak II.

Step 8: Second Ammonium Sulfate Fractionation. The main protein fractions of peak II (tubes 96 to 120) were pooled, and myosin was precipitated in a 35–55% ammonium sulfate fraction as described in step 7. The precipitate was dissolved in a solution containing 0.6 M KCl, 5 mM Tris-HCl buffer, pH 7.5, and 1 mM dithiothreitol, and it was dialyzed against a large excess of the same solution.

This method gives homogeneous myosin, free of actin and other proteins, as can be seen on the $NaDodSO_4$ gel shown at the top of peak II (Figure 1). Myosin obtained by this method is usually quite stable for 3–4 weeks at 5 °C if at least 0.5 mM dithiothreitol is present.

Enzyme Assay. The ATPase activity was determined in a medium containing 0.6 M KCl, 50 mM Tris-HCl, pH 7.3, and 10 mM $CaCl_2$. ATP was added as indicated. Departures from these conditions are expressed in the legends. All kinetic determinations were carried out for 2–3 min at 25 °C. The total protein added ranged between 10 and 20 μg. Under these conditions, the ATPase reactions were linear (Figures 2A and 2B). After the necessary incubation at 25 °C, the reaction was stopped with cold 10% Cl_3CCOOH. The tubes were centrifuged and the inorganic phosphate in the supernatant solution was determined by the method of Chen et al. (1956). This method, with minor adaptation, can accurately detect as little as 1 nmol of phosphate and allows measurement of ATP hy-

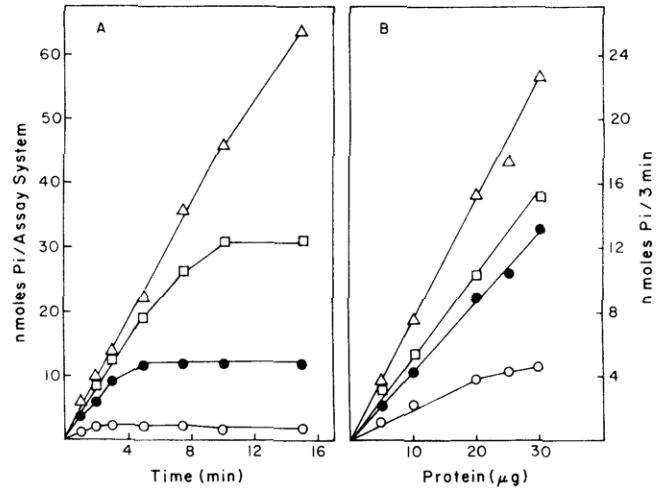

FIGURE 2: Effect of incubation time (A) and enzyme concentration (B) on the reaction rate. Activities were measured as described under Experimental Procedure. For A, 20 μg of protein was used at four different ATP concentrations: (O—O) 3.5 μM ATP; (●—●) 20 μM ATP; (□—□) 50 μM ATP; (Δ—Δ) 1.0 mM ATP. For B, the reaction was stopped after 3 min. The ATP concentrations were (O—O) 7.1 μM ATP; (●—●) 71 μM ATP; (□—□) 500 μM ATP; (Δ—Δ) 1.0 mM ATP.

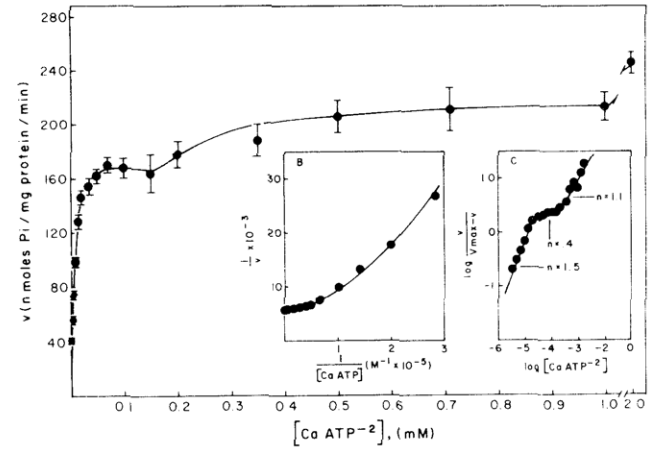

FIGURE 3: Initial reaction velocity as a function of ATP concentration. Activities were measured as described under Experimental Procedure. The error bars indicate the average deviations of eight or nine determinations for each point. Inset B is a double-reciprocal plot for substrate concentrations up to 0.15 mM and inset C is a Hill plot for all points.

[1] Abbreviations used: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; ATP, adenosine triphosphate; $NaDodSO_4$, sodium dodecyl sulfate.

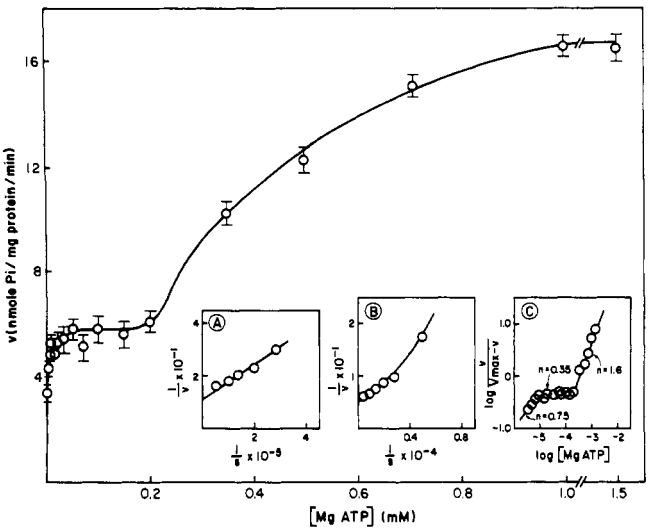

FIGURE 4: Dependence of reaction velocity of myosin on ATP concentration. Activities were measured as described under Experimental Procedure except that 2.0 mM $MgCl_2$ replaced the $CaCl_2$. The error bars indicate the average deviations of five or six determinations for each point. Inset A is a Lineweaver and Burk plot for substrate concentrations below the plateau. (B) Double reciprocal plot above the plateau. (C) Hill plot of the rate data.

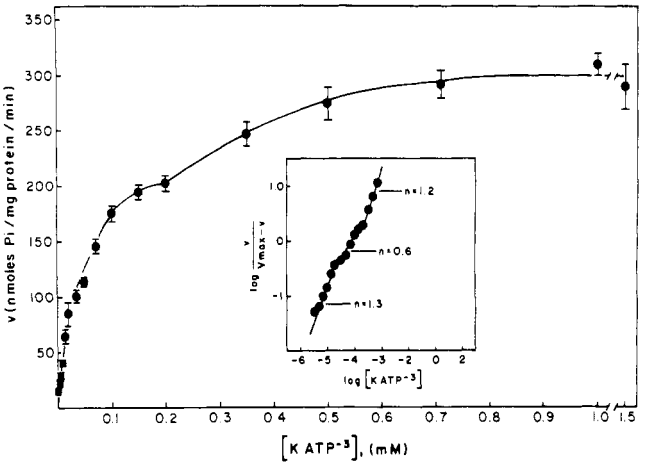

FIGURE 5: Dependence of reaction velocity of myosin on ATP concentration. Activities were measured as described under Experimental Procedure except that 2.5 mM EDTA replaced $CaCl_2$. The inset shows the Hill plot of the rate data.

drolysis without recourse to a radioactive nucleotide. The experiments reported in Figures 3 and 4 were also conducted using a creatine kinase ATP regenerating system according to Pemrick and Weber (1976). The reaction medium contained 0.6 M KCl, 50 mM Tris-HCl, pH 7.3, 5 mM creatine phosphate, 0.2 mg/mL creatine kinase and 20 μg of myosin. The other conditions are expressed in the legends. The ATPase activities of the enzyme did not differ more than 10% from preparation to preparation and gave very reproducible results. The data reported in each figure represent means of at least eight determinations at each ATP concentration with the standard deviations. Error limits are omitted in inverse and Hill plots for clarity.

Analytical Gel Electrophoresis. This was done according to the method of Weber and Osborn (1969). Protein samples were dissociated by dialyzing for 2–3 h at 25 °C in a solution containing 2% sodium dodecyl sulfate and 2% β-mercaptoethanol before electrophoresis. Protein concentration was determined by the modified Folin method of Lowry et al. (1951).

Materials

Mes and dithiothreitol were purchased from Sigma Chemical Co., St. Louis, Mo. Sepharose 4B was the product of Pharmacia Fine Chemicals, AB Uppsala, Sweden. ATP was purchased from P-L Biochemicals, Inc.

Results

Figure 1 is an elution profile of gizzard myosin (prepared according to the method described under Experimental Procedure) on a Sepharose 4B column and shows gels for each peak. Peak 1, which was eluted near the exclusion limit of the column, includes heavy chains of myosin, actin, and other impurities. Due to the low concentration of the sample, light chains of myosin are not obvious here but have been observed in similar experiments. Most of the material eluted in peak II is recognized as myosin (with one heavy and two light chains) and is free of any actin. This material was used in all of the kinetic studies described in this report.

Effect of ATP Concentration. Figure 3 shows initial activities as a function of ATP concentration. The activity change occurs in two phases which are separated by an apparent plateau which ranges from 0.05 mM to 0.15 mM Ca-ATP. This effect is highly reproducible with different preparations of the enzyme. A similar kinetic pattern was obtained when the rate of ATPase activity was determined using a creatine kinase ATP regenerating system. The Lineweaver and Burk plot is nonlinear (Figure 3, inset B) and a Hill plot of the data (inset C) gave a value of $n = 1.5$ for the section of the curve below the plateau, suggesting positive cooperativity, and a value of $n = 1.1$ for the section of the curve above the plateau. The value of $n = 0.4$ in the intermediate substrate level may be indicative of a negative cooperative effect (Cornish-Bowden and Koshland, 1975; Levitzki and Koshland, 1969). Similar results were obtained at low salt (not shown). No difference in the pattern of substrate saturation curves was found when the rate measurements were made at 5 °C either at high or at low salt (not shown). Similar experiments with skeletal muscle myosin gave hyperbolic kinetics.

Effect of Divalent Cations on Substrate Saturation Kinetics. The specific ATPase activity[2] (expressed in nmol of P_i per mg of protein per min at 25 °C) of myosin differs with each divalent cation, these being: Ca^{2+} (220 ± 20), Mg^{2+} (15 ± 5), Mn^{2+} (120 ± 15), Sr^{2+} (120 ± 12), Ba^{2+} (100 ± 10), Co^{2+} (6 ± 4), Zn^{2+} (8 ± 3), EDTA (280 ± 15); but Figure 4 shows that, while substitution of Mg^{2+} for Ca^{2+} decreases V_{max} by an order of magnitude, it does not change the characteristic of the substrate saturation curve. Addition of 10 μM Ca^{2+} in the presence of Mg^{2+} had no effect on the saturation curve (not shown). Essentially the same results were obtained by using the ATP regenerating system. Similar effects were observed when Mn^{2+} was used as the activator (not shown). In addition, removal of divalent cation with EDTA, which gives maximum activity (see above), resulted in substantially the same curve except with a less pronounced plateau (Figure 5).

Effect of pH on the Substrate Saturation Kinetics. The ATPase activity of myosin is strongly dependent on pH (Figure 6). The pH dependence of the substrate saturation curves is shown in Figure 7. For comparison, the rate data at pH 7.3 is also included (Figure 7, lower curve). At either pH 9.0 or 5.5,

[2] The ATPase activities were measured as described under Experimental Procedures. Divalent cations and EDTA were 2.5 mM and ATP was 0.5 mM. The numbers in parentheses indicate the standard error of four measurements.

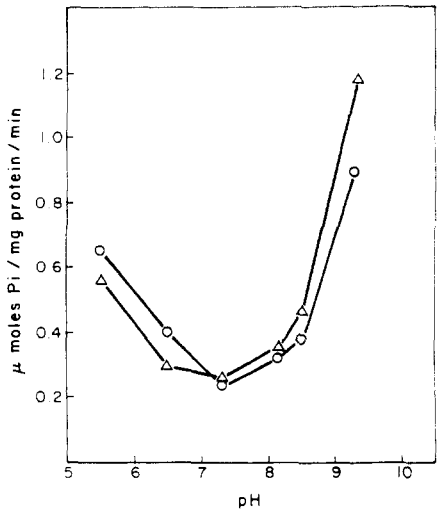

FIGURE 6: The pH dependence of Ca^{2+} activated ATPase activity of gizzard myosin. The activities were measured as described under Experimental Procedure except that ATP was 1.0 mM. Other conditions were: (O—O) in the presence of 0.6 M KCl; (Δ—Δ) at 0.15 M KCl. The incubation mixtures contained 0.05 M buffer (Mes for pH 5.5, imidazole for pH 6.2, and Tris-HCl for pH 7.3 to 9.2).

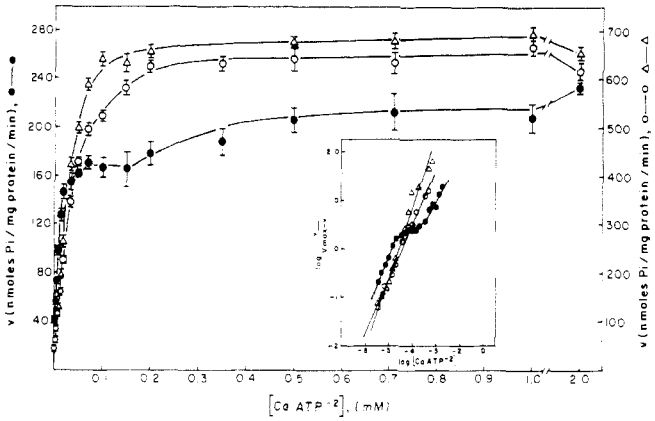

FIGURE 7: Variation of the activity of gizzard myosin with ATP concentration at different pH values. Assay conditions were as described under Experimental Procedure. (Δ—Δ) pH 5.5; (O—O) pH 9.0; (●—●) pH 7.3. The inset shows the Hill plots of the rate data at each pH. The error bars indicate the average deviations of five or six determinations for each point.

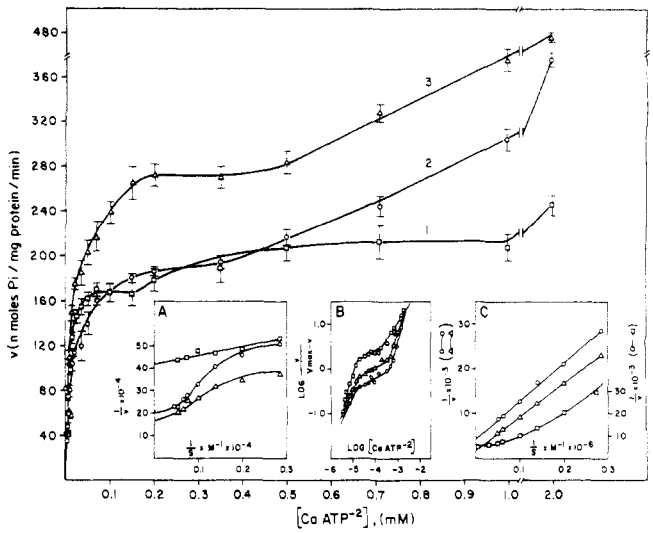

FIGURE 8: Substrate saturation kinetics of heated and urea-treated myosin. Assay conditions were as described under Experimental Procedure. (1 and □—□) Native enzyme; (2 and O—O) enzyme heated for 1.5 min at 49 °C and cooled before assay; (3 and Δ—Δ) in the presence of 1.0 M urea. The enzyme was also reacted with 1.0 M urea for 30 min *in ice prior* to assay. Inset: (A) double-reciprocal plots above the plateau for curves 1, 2, and 3. (B) Hill plots of the rate data for curves 1, 2, and 3. (C) Double-reciprocal plots below the plateau for curves 1, 2, and 3.

no plateau in the velocity curve is observed and Hill plots (inset) gave a slope of 1.5 for pH 5.5, indicating at least 2 interacting binding sites, and 1.1 for pH 9.0, suggesting no (or greatly diminished) cooperativity of binding. In addition, enzyme activity at both the higher and lower pH was slightly inhibited at 2 mM CaATP.

Effect of Heat and Urea. It is known that many enzymes possessing cooperative binding properties are sensitive to heat and urea (Monod et al., 1963; Gerhart and Pardee, 1962). Therefore, the substrate saturation kinetics of myosin was investigated using either heated or urea treated enzyme. The plateau region in the case of heated (Figure 8, curve 2) or urea treated enzyme (Figure 8, curve 3) shifts to higher substrate concentrations (compare with control, Figure 8, curve 1). The activities at low substrate concentrations with urea treated enzyme are substantially higher (Figure 8, curve 3) when compared with either control or heated enzyme. The effect of heat and urea on the enzyme is striking in that the V_{max} is doubled. The double-reciprocal plots below the plateau region (Figure 8, inset C) in the case of heated or urea treated myosin are linear in contrast to the control enzyme. The Hill plot of these data (Figure 8, inset B) gave values of $n = 1.5$ for the control enzyme below the plateau. Above the plateau region, the double-reciprocal plots for heated or urea treated enzyme are nonlinear (Figure 8, inset A) in contrast to the control, and the Hill plot for these data yielded values of $n = 1.8$ (Figure 8, inset A) for both heated and urea treated enzyme but only 1.0 for the control. At the intermediate substrate level, the values of Hill coefficient $n = 0.45$ for curves 1, 2, and 3 (Figure 8, inset B) clearly indicate the presence of a negative cooperative effect.

These observations suggest that, when the enzyme is subjected to heat or urea, cooperativity is lost at low substrate concentrations (below the plateau) but is instead seen at higher substrate concentrations (above the plateau). In addition, the negative cooperativity of binding sites does not seem to be influenced by using either the heated or the urea treated enzyme.

Discussion

Smooth muscle gizzard myosin exhibits complex substrate saturation kinetics with an intermediary plateau region. The occurrence of the plateau is a reproducible effect with different preparations of the enzyme. Although observed for the first time with smooth muscle myosin, these plateau regions have been found with several other enzymes (e.g., CTP synthetase (Levitzki and Koshland, 1969), phosphoenolpyruvate carboxylase (Corwin and Fanning, 1968), L-threonine dehydratase (Kagan and Dorozhko, 1973), and glutaminase B (Prusiner et al., 1976) from *E. coli*; glutamate dehydrogenase from *Blastochadiella* (LeJohn and Jackson, 1968), glyceraldehyde-3-phosphate dehydrogenase from honeybees (Gelb et al., 1970); DT diaphorase from liver (Ernster et al., 1972); and 15-hydroxyprostaglandin dehydrogenase (Bardsley and Crabbe, 1976)).

I have considered the possibility that the unusual kinetic behavior of smooth muscle myosin might possibly be due to minor impurities (e.g., a kinase that modified myosin and that

was active only at the higher ATP concentrations). This seems unlikely, because the kinetic properties are essentially the same with a crude actomyosin preparation or with a highly purified myosin and because they have been highly consistent with all purified enzyme preparations. In addition, plots of velocity vs. enzyme concentrations are shown to be linear over the range of enzyme concentrations used at four different substrate concentrations (Figure 2B), indicating that the enzyme is not undergoing association-dissociation reactions. I have also considered whether the observed effects are due to artifacts arising from changes in pH or ionic strength. Under the conditions of assay we could detect no change in the pH of the reaction mixture, and changes in ionic strength due to addition of substrate were negligible since the concentration of the buffer was always several orders of magnitude greater than that of substrate.

The molecular events that may explain the complex kinetic behavior of smooth muscle myosin might be described by considering the rate analysis given by Teipel and Koshland (1969). The plateau regions will only be produced when the enzyme possesses more than two substrate binding sites and the relative magnitude of the intrinsic catalytic or binding constant of these sites first decreases, then increases, as the enzyme is saturated. The data presented here in the form of Hill plots (Figures 3–5) does indeed suggest the presence of four interacting substrate binding sites.

Teipel and Kosland (1969) also pointed out that the decreased affinity for substrate could be due to either a ligand induced conformational change or a mixture of two or more proteins or subunits, each with a different intrinsic binding constant. Myosin used in this work appears to be quite homogeneous (Figure 1) and is the only protein responsible for the observed phenomena. The data reported here may result from ligand induced conformational changes. However, the possibility of any heterogeneity in the myosin molecule was also considered as a possible cause for the observed complex kinetic behavior but more information about the detailed structure of smooth muscle myosin is required before entertaining this notion. In the absence of a divalent cation (Figure 5), the extent of the plateau is reduced and the Hill plot suggests cooperative interactions are weak under these conditions (Levitzki and Koshland, 1969).

The cooperativity of the substrate binding sites is affected by changes in pH. The elimination of the plateau at pH 9.0 and 5.5 (Figure 8) suggests the absence of a negative cooperative effect. A similar disappearance of the plateau by changes in pH was also observed by Kagan and Dorozhko (1973). The lack of cooperativity of binding sites at pH 9.0 in contrast to pH 5.5 may be due to ineffective $Ca\text{-}ATP^{2-}$ binding or to a desensitization phenomenon (Monod et al., 1963). The influence of pH on the cooperativity of binding sites has been noted in a number of other enzyme systems (Wyman, 1948; Gerhart and Pardee, 1963; Rozengurt et al., 1969) including platelet myosin (Malik and Stracher, 1977).

When either the heated or urea treated enzyme was used, the kinetic properties were substantially modified: (i) the plateau region was shifted to higher substrate concentrations; and (ii) cooperativity was lost at low substrate concentrations but was still observed at higher concentrations (Figure 8). Interestingly, the shifting of the plateau region by prior heating of the enzyme has recently been reported (Prusiner et al., 1976), and the loss of cooperativity of substrate binding sites by using either heated or urea treated enzyme has been noted on other well studied enzyme systems (Monod et al., 1963; Gerhart and Pardee, 1962) and more recently by us on platelet myosin (Malik and Stracher, 1977). The persistence of negative cooperativity of smooth muscle myosin during treatment by heat and urea is in fact surprising.

The nature and cooperativity of substrate binding sites of skeletal muscle myosin, which is an extensively investigated system, have been subjects of current interest. It is generally believed that there are two substrate binding sites (Eisenberg and Moos, 1970; Schliselfeld and Bafany, 1968) and cooperativity between these sites has also been postulated (Lymn and Taylor, 1970; Kiely and Martonosi, 1968; Pemrick and Walz, 1972; Walz, 1973). In spite of the generally held view of only two substrate binding sites, Wagner and Yount (1975) using a substrate analogue reported two additional binding sites; Bowen and Evans (1968) demonstrated 14 nonactive binding sites.

While this manuscript was in preparation, a report by Nihei and Filipenko (1975) was brought to the attention of the author where they investigated the steady-state kinetic behavior of skeletal muscle myosin. On comparing the present data with their study in the presence of Mg-ATP at 0.6 M KCl, the following differences have been noted: (1) the plateau observed on the saturation curve (this work) is quite pronounced and has been reproduced on at least seven or eight different enzyme preparations; (2) the cooperativity of substrate binding sites occurs above the plateau, i.e., at higher Mg-ATP concentrations (this work). In contrast Niehei and Filipenko (1975) observed the cooperativity at low ATP concentrations. In addition, using a substrate analogue, they also obtained 12 nonspecific binding sites. The kinetic behavior of smooth muscle myosin in the presence of Ca-ATP cannot be compared with that of Nihei and Filipenko (1975) because they did not use Ca-ATP as the substrate. However, during the course of the present investigation, control experiments with skeletal muscle myosin in the presence of Ca-ATP as the substrate gave hyperbolic kinetics in contrast to the complex kinetic behavior observed with smooth muscle myosin.

The data obtained in the presence of Mg^{2+} (Figure 4) is very surprising in that the K_m is about 3×10^{-4}, whereas the K_m for skeletal muscle myosin is about 10^{-7} (Waltz, 1973). One wonders if the K_m value has any meaning in a complex kinetic situation as is the case with smooth muscle myosin. In any event, the reason for this difference is not clear at the present time. It may be that Mg-ATP is a poor substrate for the smooth muscle myosin. More detailed information on the number and nature of these binding sites using direct equilibrium methods is required before any mechanism is proposed. Such studies are currently under investigation in this laboratory.

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References

Bafany, M., Bafany, K., Gaetjens, E., and Bailin, G. (1966), *Arch. Biochem. Biophys. 113,* 205.

Bardsley, W. G., and Crabbe, M. J. C. (1976), *Eur. J. Biochem. 68,* 611.

Bowen, W. J., and Evans, T. C., Jr. (1968), *Eur. J. Biochem. 5,* 507.

Chen, P. S., Jr., Toribara, T. Y., and Warner, H. (1956), *Anal. Chem. 28,* 1756.

Cohen, I., Kaminski, E. K., Lamed, R., Oplatka, A., and Muhlard, A. (1976), *Arch. Biochem. Biophys. 175,* 249.

Cornish-Bowden, A., and Koshland, D. E., Jr. (1975), *J. Mol.*

Biol. 95, 201.
Corwin, L. W., and Fanning, G. K. (1968), *J. Biol. Chem. 243,* 3517.
Eccleston, J. F., Geeves, M. A., Trentham, D. R., Bagshaw, C. R., and Mrwa, U. (1976), in Molecular Basis of Motility, Heilmeyer, L. M. G., Ruegg, J. C., and Wieland, Th., Ed., New York, N.Y., Springer-Verlag, p 42.
Eisenberg, E., and Moos, C. (1970), *Biochemistry 9,* 4106.
Ernster, L., Lind, C., and Rose, B. (1972), *Eur. J. Biochem. 25,* 198.
Gelb, W. G., Oliver, E. J., Brandts, J. F., and Nording, J. H. (1970), *Biochemistry 9,* 3228.
Gerhart, J. C., and Pardee, A. G. (1962), *J. Biol. Chem. 237,* 891.
Gerhart, J. C., and Pardee, A. B. (1963), *Cold Spring Harbor Symp. Quant. Biol. 28,* 491.
Huxley, H. E. (1973), *Nature (London) 243,* 445.
Kagan, Z. S., and Dorozhko, A. I. (1973), *Biochim. Biophys. Acta 302,* 110.
Kiely, B., and Martonosi, A. (1968), *J. Biol. Chem. 243,* 2273.
LeJohn, H. B., and Jackson, S. (1968), *J. Biol. Chem. 243,* 3447.
Levitzki, A., and Koshland, D. E., Jr. (1969), *Proc. Natl. Acad. Sci. U.S.A. 62,* 1121.
Lowry, O. H., Rosebrough, N. J., Farr, A., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.
Lymn, R. W., and Taylor, E. W. (1970), *Biochemistry 9,* 2975.
Malik, M. N., Abramowitz, J., Detwiler, T. C., and Stracher, A. (1974), *Arch. Biochem. Biophys. 161,* 268.
Malik, M. N., Detwiler, T. C., and Stracher, A. (1973), *Biochem. Biophys. Res. Commun. 61,* 1071.
Malik, M. N., and Stracher, A. (1977), *Arch. Biochem. Biophys. 178,* 451.
Monod, J., Changeux, J. P., and Jacob, F. (1963), *J. Mol. Biol. 8,* 306.
Nihei, T., and Filipenko, C. A. (1975), *Can. J. Biochem. 53,* 1282.
Pemrick, S. M., and Walz, Jr., F. G. (1972), *J. Biol. Chem. 247,* 2959.
Pemrick, S., and Weber, A. (1976), *Biochemistry 15,* 5193.
Prusiner, S., Davis, J. N., and Stadtman, E. R. (1976), *J. Biol. Chem. 251,* 3447.
Rozengurt, E., Asua, L. J. D., and Carminatti, H. (1969), *J. Biol. Chem. 244,* 3142.
Schliselfeld, L. H., and Bafany, M. (1968), *Biochemistry 7,* 3206.
Taylor, E. W., and Sleep, J. A. (1976), *Cold Spring Harbor Symp. Quant. Biol. 3,* 127.
Tiepel, J., and Koshland, D. E., Jr. (1969), *Biochemistry 8,* 4656.
Trentham, D. R., Eccleston, J. F., and Bagshaw, C. R. (1976), *Rev. Biophys. 9,* 217.
Wagner, P. D., and Yount, R. (1975), *Biochemistry 14,* 1900.
Walz, Jr., F. G. (1973), *J. Theor. Biol. 41,* 357.
Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244,* 4406.
Wyman, J. (1948), *Adv. Protein Chem. 4,* 407.