Fukuda, M., Lauffenburger, M., Sasaki, H., Rogers, M. E., & Dell, A. (1987) *J. Biol. Chem. 262*, 11952–11957.
Furthmayr, H. (1978) *J. Supramol. Struct. 9*, 79–95.
Hamaguchi, H., & Cleve, H. (1972) *Biochim. Biophys. Acta 278*, 271–280.
Hoare, D. G., & Koshland, D. E. (1967) *J. Biol. Chem. 242*, 2447–2453.
Kathan, R. H., Winzler, R. J., & Johnson, C. A. (1961) *J. Exp. Med. 113*, 37–45.
Klenk, E., Faillard, H., & Lempfrid, H. (1955) *Hoppe-Seyler's Z. Physiol. Chem. 301*, 235–246.
Lange, L. G., Riordan, J. F., & Vallee, B. L. (1974) *Biochemistry 13*, 4361–4370.
Lisowska, E., & Duk, M. (1975) *Eur. J. Biochem. 54*, 469–474.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.
Marchesi, V. T., & Andrews, E. P. (1971) *Science 174*, 1247–1248.
Pacitti, A. F., & Gentsch, J. R. (1987) *J. Virol. 61*, 1407–1415.
Pardoe, I. U., & Burness, A. T. H. (1981) *J. Gen. Virol. 57*, 239–243.
Paul, R. W., & Lee, P. W. K. (1987) *Virology 159*, 94–101.
Paul, R. W., Choi, A. H., & Lee, P. W. K. (1989) *Virology 172*, 382–385.
Reuter, G., Stoll, S. Kamerling, J. P. Vliegenthart, J. F. G., & Schauer, R. (1988) in *Sialic Acids 1988, Procedings of the Japanese–German Symposium on Sialic Acids* (Schauer, R., & Yamakawa, T., Eds.) pp 88–89, Kieler Verlag Wissenschaft + Bildung, Kiel.
Ryzewski, C. N., & Pietruszko, R. (1977) *Arch. Biochem. Biophys. 183*, 73–82.
Schauer, R. (1987) *Methods Enzymol. 138*, 132–161.
Suttajit, M., & Winzler, R. J. (1971) *J. Biol. Chem. 246*, 3398–3404.
Tomita, M., & Marchesi, V. T. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2964–2968.
Warren, L. (1959) *J. Biol. Chem. 234*, 1971–1975.
Wiley, D. C., & Skehel, J. J. (1987) *Annu. Rev. Biochem. 56*, 365–394.
Yankeelov, J. A. (1972) *Methods Enzymol. 25*, 566–579.

Electrostatic Contributions to the Binding of Myosin and Myosin–MgADP to F-Actin in Solution†

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ABSTRACT: The ionic strength dependence of skeletal myosin subfragment 1 (S1) binding to unregulated F-actin was measured in solutions containing from 0 to 0.50 M added lithium acetate (LiOAc) in the absence and presence of MgADP. The data were analyzed by using a theory based on an ion interaction model that is rigorous for high ionic strength solutions [Pitzer, K. S. (1973) *J. Phys. Chem. 77*, 268–277] in order to obtain values for K, the equilibrium association constant when the ionic strength is zero, and for $|z_M z_A|$, the absolute value of the product of the net electric charges of the actin binding site on myosin (z_M) and the myosin binding site on actin (z_A). The presence of MgADP reduced K by a factor of 10, as expected, and reduced $|z_M z_A|$ by about 1 esu^2. Because the presence of MgADP is not likely to change the net charge of the myosin binding site on actin, these data are consistent with a model in which MgADP binding to S1 reduces its affinity for actin by a mechanism that reduces the net electric charge of the acting binding site on S1. The value of $|z_M z_A|$ in the absence of ADP was 8.1 ± 0.9 esu^2, which, if one uses integer values, suggests that z_M and z_A are in the 8+ to 1+ esu and 1− to 8− esu ranges, respectively. ADP binding then reduces z_M to the 7+ to 0.88+ esu range.

In the absence of regulatory proteins, the interactions at the interface of actin and myosin are determined primarily by the actin binding site on myosin, which in turn is controlled by the structure of the ligand that occupies the distant myosin nucleotide binding site. Chemomechanical energy transduction by muscle comprises the details of the mechanism of this control, which are not well understood. One facet of the transduction mechanism consists of the changes in the affinity of myosin for actin that are caused by changes in the ligand bound at the myosin nucleotide binding site. The standard free energy change for myosin subfragment 1 (S1)[1] binding to F-actin in solution at 25 °C and 140 mM ionic strength is increased 3–4-fold as S1-bound MgATP is hydrolyzed and then dissociates from the acto–S1 complex during the ATP hydrolysis cycle (Lymn & Taylor, 1971). In muscle, mechanical force is generated by the acto–S1–nucleotide complex as it forms and stabilizes. This coupling of increased binding energy and force generation makes the details of the bound nucleotide-induced structural changes at the actin binding site on S1 as they relate to actin binding of interest. Two broad approaches have been used to investigate the acto–S1 interface and its modification by S1-bound nucleotides. One is the

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[1] Abbreviations: S1, myosin subfragment 1; OAc, acetate; z_M, net electric charge of the actin binding site on myosin; z_A, net electric charge of the S1 binding site on actin; K_{app}, apparent association constant; K, association constant in zero ionic strength; I, ionic strength; a, activity; m, molarity; c, concentration; γ, activity coefficient; b, α, $\beta(0)$, $\beta(1)$, and C, constants in the Pitzer (1977) treatment of ions in solution.

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identification of the S1 and actin primary sequences that are at the acto–S1 interface when different ligands are bound to S1 (Yamamoto & Sekine, 1979a,b; Mornet et al., 1981, 1984; Botts et al., 1982; Sutoh, 1982, 1983; Chen et al., 1985; Bonet et al., 1988; Chausspied & Morales, 1988; Yamamoto, 1989; Chausspied, 1989). The other is the application of the techniques of solution chemistry to characterize the nature of the acto–S1 binding when different ligands are bound to S1.

When the former approach has provided the surface contours and localized charge densities of the binding sites, it will be possible to determine electrostatic contributions to binding energies for all conditions by using the finite difference Poisson–Boltzmann method (Warwicker & Watson, 1982; Sharp & Honig, 1990). Regarding the latter approach, attempts have been made to use ionic strength dependencies of acto–S1 binding to resolve the electrostatic contributions. The ionic strength effect on actomyosin binding is well established (Portzehl et al., 1950; Tonomura et al., 1962; Takeuchi & Tonomura, 1971; Beinfeld & Martonosi, 1975; Marston & Weber, 1975; Margossian & Lowey, 1978; Greene, 1981; Criddle et al., 1985; Duong & Reisler, 1987), and several systematic investigations of the ionic strength dependency of acto–S1 binding have been made (Highsmith, 1977; Wadzinski et al., 1979; Greene et al., 1983; Chalovich et al., 1984; Katoh & Morita, 1984). However, the interpretation of results has been limited by the lack of adequate theory to analyze data from measurements made by using solutions at the relatively high ionic strengths usually required for protein stability and enzymatic activity. The original theory of Debye and Huckel (1923a,b) is only accurate at less than 0.010 M ionic strength. The several phenomenologically modified theories for higher ionic strengths [see, for example, Friedman (1960)] do not allow unambiguous interpretations because of the assumptions involved. However, more recent advances in the theory of electrolyte solutions have been made by using an ion interaction model (Pitzer, 1973, 1977, 1979). This treatment is valid for solutions of mixed electrolytes (Pitzer & Kim, 1974) and makes it possible to analyze data for solutions with ionic strengths in and above the ranges used for biological studies. The measurements reported here for skeletal muscle proteins are of apparent equilibrium constants (K_{app}) for the reaction unregulated actin + S1 $\rightleftharpoons$ acto–S1, at 25 °C and pH 7.0, in solutions containing increasing amounts of lithium acetate (LiOAc) in the absence and presence of MgADP. The data are analyzed by using the theory of Pitzer (1973, 1979) in order to determine the absolute value of the product of the net electrical charges of the binding sites on S1 and actin, $z_M z_A$, and of the equilibrium constant at 0 M ionic strength.

Materials and Methods

Chemicals and Proteins. All chemicals were reagent grade or better. The stock solution concentration of LiOAc was adjusted so that when diluted to 0.010 M in double-distilled water, it had a conductance equal to that of standard 0.010 M KCl (Ricca Chemical Co.). The ionic strength was calculated from $I = \sum^1/_2(m_i z_i^2)$ where z_i is the electric charge and m_i is the molar concentration of each of the i ionic species present.

Myosin was isolated from rabbit skeletal muscle (Nauss et al., 1969) and used to prepare S1 (Margossian & Lowey, 1978). S1 was also prepared from myofibrils (Cooke, 1972). In either case, papain was used in the presence of 1–5 mM $MgCl_2$. S1 was purified by size-exclusion chromatography, using Sephacryl S-400. MgATPase activities at 25 °C were typically 0.044 s^{-1} for S1 and 1.06 s^{-1} for acto–S1. *N*-Acetyl-*N'*-(5-sulfo-1-naphthyl)ethylenediamine-labeled S1 (AEDANS-S1) was prepared (Duke et al., 1976; Highsmith et al., 1976), and unattached fluorescent label was removed by dialysis. AEDANS-S1 samples were centrifuged at 80000*g* for 1 h before use. F-Actin was prepared as described by Spudich and Watt (1971) from acetone-extracted muscle tissue (Feuer et al., 1948).

Association Constants. The method of Marston and Weber (1975) was used to measure the fractions of bound and free S1 in the presence of F-actin except that AEDANS-S1 fluorescence was used to detect S1 instead of the radioactivity of [^{14}C]acetamide-labeled S1. The AEDANS is attached to Cys-707, which does not appear to alter the affinity of S1 for F-actin (Highsmith et al., 1976; Yoshimura & Mihashi, 1982). In any event, the primary purpose of the present study is to detect changes in the affinity caused by nucleotide binding to S1, so label effects that might change absolute values will not affect the conclusions, which depend on changes of ratios.

Data Analysis. The reversible equilibrium binding of the solutes M and A

$$M + A \rightleftharpoons MA \quad (1)$$

can be described by the expression

$$K = a_{MA}/a_M a_A = (c_{MA}/c_M c_A)(\gamma_{MA}/\gamma_M \gamma_A) \quad (2)$$

where a is chemical activity, c is concentration, and γ is the activity coefficient (Moore, 1962). The ratio of the concentrations is K_{app}, the apparent association constant, which usually is measured. For ions M and A binding in solution, $\gamma_{MA} = 1$ and $\gamma_M \gamma_A$ is represented as $\gamma_\pm$, which has values that decrease from 1 as the ionic strength increases. Thus, eq 2 can be written

$$\ln K = \ln K_{app} - \ln \gamma_\pm \quad (3)$$

which describes the lowered K_{app} in terms of K at ionic strength = 0 and the activity coefficient of the ions. The greater the charges on M and A, the stronger is the dependence of K_{app} on the ionic strength of the solution.

For conditions of very low ionic strength (I), the Debye–Huckel approximation (eq 4) is as accurate as most existing measured data. It is usually given as

$$\log \gamma_\pm = -0.509|z_M z_A| I^{1/2} \quad (4)$$

for ions M and A with charges z_M and z_A, respectively. For $I < 0.010$ M, eq 3 and 4 can be combined to analyze measurements of K_{app} at different I and obtain accurate values for K and $|z_M z_A|$. For $I > 0.010$ M, the approximations used to derive eq 4 are not valid. Several modified versions of eq 4 give good fits to data (Friedman, 1960) but are not based on rigorous theory and cannot be interpreted unambiguously.

The theory developed by Pitzer (1973a,b, 1977) has the expression relating $\gamma_\pm$ and I:

$$\ln \gamma_\pm = -0.392|z_M z_A|[I^{1/2}/(1 + bI^{1/2}) + (2/b) \ln (1 + bI^{1/2})] + m\{2\beta(0) + [2\beta(1)/\alpha^2 I][1 - (1 + \alpha I^{1/2} - \alpha^2 I/2) \exp(-\alpha I^{1/2})]\} + m^2(3C/2) \quad (5)$$

where $b = 1.2$ and $\alpha = 2.0$ for all electrolytes and $\beta(0)$, $\beta(1)$, and C are parameters that are specific for particular electrolytes. Equation 5 can be used with eq 3 to fit data from measurements of K_{app} that are made over any range of I in order to obtain estimates of the values of K and $|z_M z_A|$ that are theoretically sound.

Some differences between the two treatments can be seen in Figure 1. The solid lines are predicted values of $\gamma_\pm$ in increasing concentrations of LiOAc when $|z_M z_A| = 4$, 9, and 16, obtained by using eq 5. Curves of this type fit existing measured and Monte–Carlo-simulated data for electrolyte

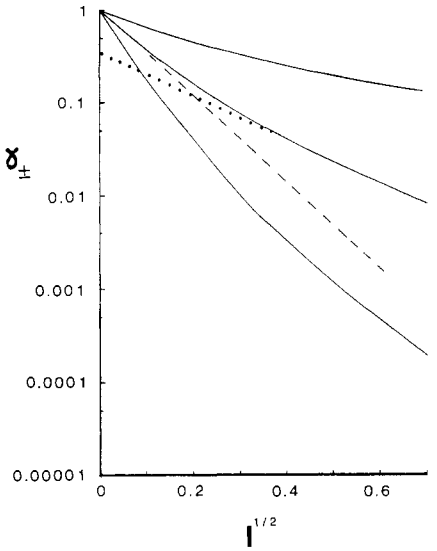

FIGURE 1: Activity coefficients as a function of ionic strength. Semilogarithmic plots of activity coefficients ($\gamma_\pm$) as a function of the square root of the ionic strength (solid lines) were obtained by using the theory of Pitzer (eq 5) for cases in which the absolute values of the product of the ionic charges are $|z_M z_A| = 4$, 9, and 16 (in decending order), and the ionic strength is adjusted with LiOAc. The dashed line was obtained by using the Debye–Huckel limiting law (eq 4) and $|z_M z_A| = 9$. The dotted line is a linear best fit to the values generated by using eq 5 in the range $0.15 < I < 0.50$ M (see text).

Table I: Values for K and $|z_M z_A|$[a]

ligand	K (M^{-1})	$\lvert z_M z_A \rvert$ (esu^2)
none	$(3.9 \pm 1.4) \times 10^8$	8.09 ± 0.80
ADP	$(3.7 \pm 1.7) \times 10^7$	7.12 ± 0.97

[a] The values for K and $|z_M z_A|$ were obtained from nonlinear least-squares best fits of eq 3 and 5 to the data shown in Figure 2 (see text).

solutions very well (Pitzer, 1977). The dashed line shows the values of $\gamma_\pm$ for $|z_M z_A| = 9$ obtained by using the Debye–Huckel limiting law (eq 4). The well-known large discrepancy at higher I is clear. However, the main point of Figure 1 is demonstrated by the dotted line which is a straight line drawn through the values predicted by eq 5 for $|z_M z_A| = 9$ in the 0.15–0.5 M range of I, and extrapolated to $I = 0$. The values in this region of I are reasonably well approximated by a straight line, especially if experimental uncertainty were present, and this is the ionic strength range typically used in biological studies. This "good-fit" to the data will lead to an understimate of $\gamma_\pm$ (and thus K) at $I = 0$ of 65% and an understimate of $|z_M z_A|$ of 30%. These errors occur when there is a satisfactory statistical fit to the data and are the reasons ionic strength dependencies of acto–S1 binding have not been interpreted quantitively in the past.

Results

The apparent association constants (K_{app}) for the binding of AEDANS-S1 to F-actin in solutions containing increasing concentrations of LiOAc were measured at 23 °C. Semilogarithmic plots of K_{app} as a function of $I^{1/2}$ are shown in Figure 2, for acto–S1 with and without ADP present. The solid lines in each case are best fits of the data to eq 3, using eq 5 to calculate ln $\gamma_\pm$ (Pitzer, 1977). K and $|z_M z_A|$ were varied to obtain the fits, using $b = 1.2$, $\alpha = 2.0$, $\beta(0) = 0.1124$, and $\beta(1) = 0.2483$ (Pitzer & Mayorga, 1973); m is molarity and $C = 0$. The values obtained for K and $|z_M z_A|$ are given in Table I. In the absence of ADP, the value for K, $(3.9 \pm 1.4) \times 10^8$ M^{-1}, is severalfold higher than a previous estimate for AEDANS-S1 and actin (Highsmith, 1977), perhaps for

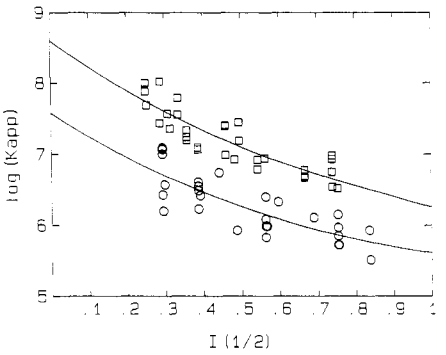

FIGURE 2: Ionic strength dependence of the observed association constant for acto–S1 binding in the presence and absence of ADP. Apparent association constants were determined for F-actin binding to AEDANS-S1 in the absence (□) and presence (○) of ADP at 23 °C in solutions which contained 5 mM K_3PO_4 (pH 7.0), 5 or 12 mM $MgCl_2$, and increasing amounts of LiOAc. Solid lines were obtained by varying the values of K and $|z_M z_A|$ in eq 5 to minimize the sum of the square of deviations from the data. The constants b, α, $\beta(0)$, $\beta(1)$, and C are given in the text.

the reason given above (see Figure 1 and Data Analysis). The K at zero ionic strength obtained for the binding of an oligopeptide that complements the presumed electric charges in the actin binding site was determined to be about 2×10^6 M^{-1} (Chausspied & Morales, 1988). The 200-fold greater K reported here for actin itself may be an estimate of the increase in acto–S1 binding that is due to nonelectrostatic interactions. The value for $|z_M z_A|$, 8.09 ± 0.97 esu^2, is reported here for the first time.

In the presence of ADP, K is reduced by a factor of 10 (Table I), and K_{app} is reduced somewhat less as the ionic strength is increased (Figure 2). Doubling the [MgADP] did not change this ionic strength dependent reduction in the ratio of K_{app} in the absence and presence of ADP. The value for $|z_M z_A|$ in the presence of ADP was 7.12 ± 0.97 esu^2.

A number of control measurements were done to reduce the possibilities that ion-specific effects of other constituents of the solutions were influencing the measurements. The fluorescence of AEDANS-S1 was constant, within experimental uncertainty, for solutions with [$MgCl_2$] ranging from 0 to 20 mM (data not shown). K_{app} was determined for AEDANS-S1 and actin, with and without ADP present, in solutions containing 0.3 M LiOAc and from 0 to 20 mM $MgCl_2$. In both cases, K_{app} decreased over this $MgCl_2$ concentration range. The decrease was accounted for by the contribution of $MgCl_2$ to the ionic strength when ADP was absent. When ADP was present, the decrease in K_{app} was larger in the 0–3 mM $MgCl_2$ range than predicted for ionic strength changes, suggesting that LiADP may not be as effective at dissociating S1 from actin as is MgADP (data not shown). This possible LiADP effect is not a problem here, because all the data shown in Figure 2 are for solutions with at least 5 mM $MgCl_2$. The effects of pH were also investigated. When compared to pH 7.0, K_{app} decreases about 40% in solutions at pH 6.0, but is constant, within experimental error, between pH 7 and 8 (data not shown). LiOAc had apparent inhibitory effects on S1 MgATP activity at concentrations above 0.3 M which were which almost completely reversed when the results were corrected for its effects on the ATP-regenerating system. Li^+ has been shown to stabilize F-actin without deleterious effects (Pan & Ware, 1988).

Discussion

The ratio of the values of K (Table I) in the absence and presence of MgADP is consistent with previous measurements

(Beinfeld & Martonosi, 1975; Highsmith, 1977; Greene & Eisenberg, 1980; Marston, 1982; Geeves & Gutfreund, 1982). The [ADP] is high enough to overcome any ionic strength dependencies of its binding to S1, in agreement with earlier work (Lowey & Luck, 1962; Hazzard & Cusanovich, 1986). The absence of a significant effect on the S1 MgATPase activity suggests that LiOAc is not affecting the nucleotide binding site, if the $[Mg^{2+}]$ is above 5 mM. The data for acto–S1 binding appear to have been collected under conditions that make it adequate for investigating the effect of MgADP binding to S1 on the electrostatic interactions at the acto–S1 interface.

In the absence of MgADP, $z_M z_A$ is 8.09. There is no previous determination to which to compare this value. It is consistent with z_M and z_A, respectively, having values varying from 8+ and 1− to 8− and 1+, using only integer values of net charge to simplify the discussion. Chemical cross-linking of actin and myosin binding sites (Sutoh, 1982, 1983; Mornet et al., 1984; Yamamoto, 1989) and of oligopeptides to those sites (Suzuki et al., 1987; Chausspied & Morales, 1988; Chausspied, 1989; Kaspzak et al., 1990) has suggested that a portion of the myosin heavy chain which contains five positive charges is part of the actin binding site and a portion of actin which contains four negative charges is part of the myosin binding site. This makes it reasonable to assign net positive and negative charge to z_M and z_A, respectively. Thus, z_M is in the 8+ to 1+ range, and z_A is in the 1− to 8− range, paired such that the absolute value of their product equals 8.

The addition of MgADP reduces $z_M z_A$ by 1 esu^2. Since it is unlikely that MgADP would affect z_A on actin, the most straightforward interpretation of this result is that MgADP binding in the myosin nucleotide binding site reduces the net electric charge z_M of the actin binding site, by from 1 to 0.12 esu, for the 8+ to 1+ range in the absence of MgADP. It is unlikely the MgADP-induced decrease in z_M is only apparent and actually reflects the trivial reduction in electric charge at the nucleotide binding site on myosin for two reasons. The first is that the actin and nucleotide binding sites on myosin are separated by about 5 nm of protein matrix (Tokugawa et al., 1987). The second reason is that surface charge changes outside a catalytic or binding site do not appear to change the electrostatic potential of the site. Finite-difference Poisson–Boltzmann calculations indicate that changing the surface charge by 12 esu does not change the electrostatic potential in the catalytic site of trypsin (Soman et al., 1989). Likewise, the "steering" and "docking" behavior during the binding of cytochrome *c* to cytochrome *c* peroxidase is determined by the electrostatic potential generated by the binding sites, not other surface charges (Northrup et al., 1987). It is more likely that MgADP binding at the nucleotide site is transmitted through the protein by a conformational change to decrease the net effective electric charge z_M at the actin binding site.

The electric charges of the amino acid side chains that have been assigned to the acto–S1 interface from chemical cross-linking studies of acto–S1 (Sutoh, 1982, 1983; Mornet et al., 1984; Yamamoto, 1989) and of an oligopeptide with S1 (Chauspied & Morales, 1988) indicate that the value of $z_M z_A$ should be 20–36 esu^2. This is larger than the value in Table I for the no ADP case, 8.09 esu^2. Although it is not necessary, it is possible to reconcile this difference. It may be that other portions of the myosin heavy or light chains contribute negatively charged side chains to the actin binding site or that other portions of the actin chain contribute positively charged side chains to the myosin binding site. It is also possible that not all of the five amino groups reputed to be in the actin binding site are protonated or that not all of the four carboxyl groups reputed to be in the myosin binding site are dissociated. It is possible that the Li^+ ion used in the experiments here binds to the myosin binding site on actin. Finally, one could assume that the electric permittivity of water should not be used in the derivation of eq 5; using a higher value would increase $z_M z_A$.

In any event, there is an internal consistency in the data presented here (Table I) that is worth pointing out. The ratio of the standard free energies of acto–S1 binding, without and with ADP, is 1.13, when $\Delta G'^\circ$ is calculated by using the experimental values of K and the equation $\Delta G'^\circ = -RT \ln K$. Ignoring any mutual polarization of the ions (Grunwald et al., 1974), the electrostatic interaction energy for ionic binding is proportional to $z_M z_A$. Thus, the ratio of the electrostatic contribution to the binding in the absence and presence of ADP is approximated by the ratio of $z_M z_A$, which is 1.14. This result suggests that at least for the step A·M·MgADP $\rightleftharpoons$ A·M + MgADP under the conditions used here, the entire increase in the affinity of myosin for actin that is due to MgADP dissociation from myosin can be quantitatively accounted for by the measured increase in electric charge on the actin binding site of myosin.

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References

Arata, T. (1984) *J. Biochem. (Tokyo) 96*, 337–347.
Beinfeld, M. C., & Martonosi, A. N. (1975) *J. Biol. Chem. 250*, 7871–7878.
Bonet, A., Audermard, E., & Mornet, D. (1988) *J. Biol. Chem. 263*, 14115–14121.
Botts, J., Muhlrad, A., Takashi, R., & Morales, M. F. (1982) *Biochemistry 21*, 6903–6905.
Chalovich, J. M., Chock, P. B., & Eisenberg, E. (1981) *J. Biol. Chem. 256*, 575–578.
Chalovich, J. M., Stein, L. A., Greene, L. E., & Eisenberg, E. (1984) *Biochemistry 23*, 4885–4889.
Chausspied, P. (1989) *Biochemistry 28*, 9123–9128.
Chausspied, P., & Morales, M. F. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 7471–7475.
Chen, T., Applegate, D., & Reisler, E. (1985) *Biochemistry 24*, 137–144.
Coates, J. H., Criddle, A. H., & Geeves, M. A. (1985) *Biochem. J. 232*, 351–356.
Cooke, R. (1972) *Biochem. Biophys. Res. Commun. 49*, 1021–1028.
Criddle, A. H., Geeves, M. A., & Jeffries, T. (1985) *Biochem. J. 232*, 343–349.
Debye, P., & Huckel, E. (1923a) *Phys. Z. 24*, 185–197.
Debye, P., & Huckel, E. (1923b) *Phys. Z. 24*, 334–348.
Doung, A. M., & Reisler, E. (1987) *J. Biol. Chem. 262*, 4129–4133.
Duke, J., Takashi, R., Ue, K., & Morales, M. F. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 302–306.
Feuer, G., Molner, F., Pettko, E., & Straub, F. B. (1948) *Hung. Acta Physiol. 1*, 150–162.
Friedman, H. L. (1960) *J. Chem. Phys. 32*, 1134–1145.
Geeves, M. A., & Gutfreund, F. (1982) *FEBS Lett. 140*, 11–15.
Greene, L. (1981) *Biochemistry 20*, 2120–2126.

Greene, L., & Eisenberg, E. (1980) *J. Biol. Chem. 225*, 543–548.
Greene, L., Sellers, J. R., Eisenberg, E., & Adelstein, R. S. (1983) *Biochemistry 22*, 530–535.
Grunwald, E., Highsmith, S., & I, T.-P. (1974) in *Ions and Ion Pairs in Organic Reactions* (Szwarc, M., Ed.) pp 447–519, Wiley, New York.
Hazzard, J. H., & Cusanovich, M. A. (1986) *Biochemistry 25*, 8141–8149.
Highsmith, S. (1977) *Arch. Biochem. Biophys. 180*, 404–408.
Highsmith, S., Mendelson, R. A., & Morales, M. F. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 133–137.
Kasprzak, A. A., Chausspied, P., & Morales, M. F. (1989) *Biochemistry 28*, 9230–9238.
Katoh, T., & Morita, F. (1984) *J. Biochem. (Tokyo) 96*, 1223–1230.
Lymn, R., & Taylor, E. D. (1971) *Biochemistry 10*, 4617–4624.
Margossian, S. S., & Lowey, S. (1978) *Biochemistry 17*, 5431–5439.
Marston, S. (1982) *Biochem. J. 203*, 453–460.
Marston, S., & Weber, A. (1975) *Biochemistry 14*, 3868–3873.
Moore, W. J. (1962) *Physical Chemistry*, 3rd ed., pp 167–202, Prentice-Hall, Englewood Cliffs, NJ.
Mornet, D., Bertrand, R., Pantel, P., Audermand, E., & Kassab, R. (1981) *Nature 292*, 301–306.
Mornet, D., Ue, K., & Morales, M. F. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 736–739.
Nauss, K., Kitagawa, S., & Gergely, J. (1969) *J. Biol. Chem. 244*, 755–765.
Northrup, S. H., Boles, J. O., & Reynolds, J. D. L. (1987) *J. Phys. Chem. 91*, 5991–5998.
Pan, X. X., & Ware, B. R. (1988) *Biophys. J. 53*, 11–16.
Pitzer, K. S. (1973) *J. Phys. Chem. 77*, 268–277.
Pitzer, K. S. (1977) *Acc. Chem. Res. 10*, 371–377.
Pitzer, K. S. (1979) in *Activity Coefficients in Electrolyte Solutions* (Pytkowicz, R., Ed.) pp 157–208, CRC Press, Boca Raton, FL.
Pitzer, K. S., & Mayorga, G. (1973) *J. Phys. Chem. 77*, 2300–2308.
Pitzer, K. S., & Kim, J. J. (1974) *J. Am. Chem. Soc. 96*, 5701–5707.
Portzehl, H., Schramm, G., & Weber, H. H. (1950) *Z. Naturforsch. 5B*, 61.
Sharp, K. A., & Honig, B. (1990) *Annu. Rev. Biophys. Biophys. Chem. 19*, 301–332.
Soman, K., Yang, A.-S., Honing, B., & Fletterick, R. (1989) *Biochemistry 28*, 9918–9926.
Spudich, J. H., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866–4871.
Sutoh, K. (1982) *Biochemistry 21*, 3654–3661.
Sutoh, K. (1983) *Biochemistry 22*, 1579–1585.
Suzuki, R., Nishi, N., Tokura, S., & Morita, F. (1987) *J. Biol. Chem. 262*, 11410–11412.
Takeuchi, K., & Tonomura, Y. (1971) *J. Biochem. (Tokyo) 70*, 1011–1026.
Tokunaga, M., Sutoh, K., Toyoshima, C., & Wakabayashi, T. (1987) *Nature (London) 329*, 635–638.
Tonomura, Y., Tokura, S., & Sekiya, K. (1962) *J. Biol. Chem. 237*, 1074–1081.
Wadzinski, L., Botts, J., Wang, A., Woodard, J., & Highsmith, S. (1979) *Arch. Biochem. Biophys. 198*, 397–402.
Warwicker, J., & Watson, H. (1982) *J. Mol. Biol. 157*, 671–679.
Yamamoto, K. (1989) *Biochemistry 28*, 5573–5577.
Yamamoto, K., & Sekine, T. (1979a) *J. Biochem. (Tokyo) 86*, 1855–1862.
Yamamoto, K., & Sekine, T. (1979b) *J. Biochem. (Tokyo) 86*, 1869–1881.
Yoshimura, H., & Mihashi, K. (1982) *J. Biochem. (Tokyo) 92*, 497–508.