Oxygen Exchange between Phosphate and Water Accompanies Calcium-regulated ATPase Activity of Skinned Fibers from Rabbit Skeletal Muscle*

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The extent of oxygen exchange between phosphate and water has been measured for the calcium-regulated magnesium-dependent ATPase activity of chemically skinned fibers from rabbit skeletal muscle. The oxygen exchange was determined for isometrically held fibers by measuring with a mass spectrometer the distribution of $^{18}$O atoms in the product inorganic phosphate when ATP hydrolysis was carried out in H$_2^{18}$O. The extent of exchange was much greater in relaxed muscle (free Ca$^{2+} < 10^{-6}$ M) than in calcium-activated muscle (free Ca$^{2+} \approx 3 \times 10^{-5}$ M). Activated fibers had an ATPase activity at least 30-fold greater than the relaxed fibers. These results correlate well with the extent of oxygen exchange accompanying magnesium-dependent myosin and unregulated actomyosin ATPase activities, respectively. In relaxed fibers, comparison of the amount of exchange with the ATPase activity suggests that the rate constant for the reformation of myosin-bound ATP from the myosin products complex is about 10 s$^{-1}$ at 20 °C and pH 7.1. In each experiment the distribution of $^{18}$O in the P$_i$ formed was incompatible with a single pathway for ATP hydrolysis. In the case of the calcium-activated fibers, the multiple pathways for ATP hydrolysis appeared to be an intrinsic property of the actomyosin ATPase in the fiber. These results indicate that in muscle fibers, as in isolated actomyosin, cleavage of protein-bound ATP is readily reversible and that association of the myosin products complex with actin promotes P$_i$ release.

In the sliding filament theory, muscle contraction is mediated by the cycling of cross-bridges that form a mechanical link between the thick and thin filaments of the sarcomere (H. E. Huxley and Hanson, 1954; A. F. Huxley and Niedergerke, 1954; A. F. Huxley, 1957; H. E. Huxley, 1969). In vertebrate striated muscle, a magnesium-dependent ATPase regulated by calcium ions is located within these cross-bridges. In order to understand the transduction of chemical energy to mechanical work, the elementary processes of the ATPase must be correlated directly with the mechanical constraints on the cross-bridges and the performance of work by the muscle. This requires techniques for probing the mechanism of the ATPase that are applicable to intact biological structures. We describe here measurements of the extent of exchange of oxygen atoms between the solvent water and phosphate during ATP hydrolysis catalyzed by the fiber. In principle, this permits the kinetics of elementary steps in the ATPase mechanism to be related to the kinetics of the cross-bridge cycle.

Oxygen exchange between phosphate and water during ATP hydrolysis catalyzed by muscle tissue was first reported by Koshland and Clarke (1953). The exchange by myosin was described by Levy and Koshland (1959) and subsequently the exchange has been analyzed extensively in studies of the ATPase (reviewed by Webb and Trentham, 1983). A minimal mechanism for the myosin ATPase is shown in Equation 1:

$$M + ATP \rightleftharpoons M \cdot ATP + H_2O \rightleftharpoons M \cdot ADP \cdot P_i$$

where $M$ represents myosin or subfragment 1, the portion of the myosin molecule that contains both actin binding and ATPase sites. It is obtained by mild proteolytic digestion of myosin (Lowey et al., 1969). Unlike myosin, subfragment 1 is soluble at low ionic strength, a requirement for maximum activation of the ATPase by actin.

Oxygen exchange occurs because the hydrolysis on the enzyme (Step 2, Equation 1) is readily reversible, M-ATP, and M-ADP-P$_i$ have a significant lifetime during ATPase activity, and P$_i$ can rotate in M-ADP-P$_i$ (Webb and Trentham, 1983). The proposed exchange mechanism for myosin is shown in Scheme 1, where the numbers relate to steps in the mechanism of Equation 1, and $^{18}$O is represented by $\bullet$.

If $k_{+1}$ and $k_{-1}$ are rate constants of the $i$th step, then the amount of exchange is controlled predominantly by the value of the ratio $R = k_{+3}/k_{-2}$. $k_{+3}$ has a value that approximately corresponds to the catalytic center activity of the ATPase and $k_{-2} = 15$ s$^{-1}$ for subfragment 1 at pH 8.0, 20 °C (Webb and Trentham, 1981). The situation is different with actomyosin or actosubfragment 1 because the rate constant of the step controlling P$_i$ release (related to $k_{+3}$) is enhanced several hundred-fold at high actin concentrations. $k_{-2}$ appears to have a similar value during subfragment 1 and actosubfragment 1 ATPase activities. Thus the extent of oxygen exchange is high for subfragment 1 but very low for actosubfragment 1 (Shukla and Levy, 1977; Sleep and Boyer, 1978).

It is thought that cross-bridge formation in active muscle to...
is analogous to M-ADP-P, binding to actin in solution (Lynn and Taylor, 1971). We have analyzed the extent of oxygen exchange in P, formed as a result of ATP hydrolysis in relaxed and Ca\(^{2+}\)-activated muscle fibers. The exchange provides a probe of the kinetics of the ATPase and cross-bridge formation, and the dependence of these kinetics on mechanical performance of the fiber.

**Experimental Procedures**

Skinned fibers from rabbit psoas muscle were prepared as described in Goldman et al. (1984a). Fibers were mounted with aluminum clips (Akers AE 801, Horten, Norway) and in most experiments stretched 5–10% above slack length. Fiber volumes were measured by light microscopy (Goldman and Simmons, 1984), while the fibers were immersed in a relaxing solution at 20–22 °C containing 15 mM ATP, 14 mM MgCl\(_2\), 10 mM EGTA, 10 mM reduced glutathione, 100 mM Tris and adjusted to pH 7.1 with KOH.

Incubations for ATP hydrolysis were conducted in solutions containing H\(_2\)^{18}O. Solutions were first made up in unlabeled water. They were lyophilized and then dissolved in H\(_2\)^{18}O (95% isotopic enrichment; from Prochem Inc., NJ). The cycle was repeated, and the \(^{18}O\)-enriched solutions stored frozen for up to 48 h before use.

For relaxed fiber experiments, four 35-μl troughs were filled with the \(^{18}O\)-enriched relaxing solution and then covered in a 2-cm deep layer of silicone oil (dimethyl polysiloxane; viscosity 10 cS, Dupont) to provide a barrier to exchange with atmospheric water. Four pairs of fibers were transferred sequentially through the first two troughs, layer of silicone oil (dimethyl polysiloxane; viscosity 10 cS, Dupont) to provide a barrier to exchange with atmospheric water. Four pairs of fibers were transferred sequentially through the first two troughs, spending 2–3 min in each, in order to wash unlabeled water from the fibers. The eight fibers were then transferred to the third trough and left at 20.0 ± 0.2 °C until approximately 10% of the ATP was hydrolyzed. The incubation time required was 2–3 h. As a control, \(^{18}O\)-enriched relaxing solution was kept for equal time in the fourth trough to determine the extent of spontaneous ATP breakdown. In some experiments fewer fibers were incubated but for a proportionately longer time.

For the Ca\(^{2+}\)-activated experiments, a single fiber was mounted as described above. The first two troughs contained a preactivating solution that was similar to the relaxing solution except that the EGTA concentration was 0.1 mM, and 9.9 mM 1,6-diaminohexane-\(N,N',N''\)-tetraacetic acid was added to maintain ionic strength (Moisescu, 1976). The fiber was then transferred to an activating solution at pH 7.1 containing 10 mM Ca-EGTA and approximately 30 μM free Ca\(^{2+}\) at 20 °C. The hydrolysis was stopped after 2–3 h at which time 10–20% of the ATP had been hydrolyzed. A control solution with no fiber was kept in an adjacent trough for this period.

Since relatively long contractions were required to accumulate enough Pi for mass spectral analysis, it was necessary to test the mechanical condition of the fibers. To establish appropriate incubation conditions for maximal Ca\(^{2+}\) activation, a small length release (1–2%) was imposed on the fiber to test the rate of tension recovery. Normal cycling of cross-bridges is indicated by rapid (≤7 s\(^{-1}\)) and complete recovery of tension (Abbott and Steiger, 1977). The medium was the same as that used in the Ca\(^{2+}\)-activated fiber experiments and the test was performed after activation for 30 min. At a physiological concentration of ATP (5 mM) the recovery was slow and often incomplete. When an ATP-regenerating system of 20 mM creatine phosphate and 1 mg ml\(^{-1}\) creatine kinase was added to the medium, or when ATP concentration was raised to 15 mM in the absence of the regenerating system, a rapid phase of tension recovery occurred (50–80% of the total) followed by complete recovery over several seconds. Ionic strength was kept constant by varying the Ca-EGTA concentration. These results suggest that the fiber core is nearly in rigor at 5 mM MgATP but cross-bridges are continuously cycling at 15 mM MgATP, so the latter condition was adopted for \(^{18}O\) exchange experiments. The slow recovery phase indicates that some ATP depletion with accumulation of ADP and P, occurred in the core of the fibers during the prolonged contractions. The regenerating system was not used because the overall catalytic center activity of the fiber was estimated from the ADP/ATP ratio and because of the relative instability of creatine phosphate. The fibers produced 153 ± 35 kN-m\(^{-2}\) isometric tension which is comparable to other reported values (Cooke and Bialek, 1979; Goldman et al., 1984b). Tension declined by ~30% by the end of the incubations which may be explained in part by P, accumulation (Brandt et al., 1982).

At the end of both relaxed and Ca\(^{2+}\)-activated incubations, the fibers were removed and aliquots of the solutions were taken for measurement of H\(_2\)^{18}O enrichment as described below. The percentage of hydrolysis was determined by measuring the ADP/ATP ratio in a 3-μl sample by high performance liquid chromatography on an anion exchange column (Whatman-SAX, 25 cm × 0.4 cm diameter). The nucleotides were eluted with 0.4 M (NH\(_4\))H\(_2\)PO\(_4\) at pH 4.7 containing 2% (v/v) methanol. The remainder of each incubation solution was diluted with unlabeled water so that any further hydrolysis would produce unlabeled P, and unlabeled carrier P, (70 nmol) was added.

P, was isolated from the incubation solutions on a column of Dowex 1 (1.5 cm × 0.8 cm diameter) in the Cl\(^-\) form and eluted with 10 mM HCl. Acid and water were removed in vacuo to leave H\(_2\)PO\(_4\), that was then eluted with ethereal diazomethane to give volatile triethyl phosphate. The distribution of \(^{18}O\) was analyzed by gas chromatography-mass spectrometry (Hackney et al., 1980) on a Hewlett-Packard 5995B instrument. Gas chromatography was performed at 80 °C with a silica column (12 m × 0.32 mm diameter) with methyl silicone liquid phase (1 μm thickness), and helium mobile phase (1 ml min\(^{-1}\)). Electron impact ionization was used and the intensities of the fragments m/e 155–163 (molecular ion minus C\(_2\)H\(_4\)) were measured.

The isotopic enrichment of the water in the incubation solutions was measured at the beginning and end of each experiment. An aliquot (10 μl) was added to dry PC15 (5 mg) in a sealed vial. After at least 1-h reaction time, the P, formed in this reaction was analyzed for \(^{18}O\) enrichment. The peaks corresponding to \(^{18}O\), \(^{18}O\), and \(^{16}O\) were used to calculate the water enrichment, since these peaks were

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The abbreviation used is: EGTA, ethylene glycol bis(\(\beta\)-aminoethyl ether)-\(N,N',N''\)-tetraacetic acid.
unaffected by spontaneous breakdown of ATP. Enrichments were in the range 93–97%.

Table I illustrates the method for handling the data from a typical relaxed fiber incubation. Line A shows the distribution of masses for $m/e$ 155 measured directly from 5 injections in the mass spectrometer. In this and the other experiments, the distributions varied by no more than 0.3% in any one fragment. In Line B the data of Line A are averaged.

Apart from the fragment with parent ion $m/e$ 155, there was also an ionized fragment with parent ion $m/e$ 153 (molecular ion minus C$_2$H$_4$) in the observed region of the mass chromatogram (see also Granath, 1976). This interfering fragment was approximately 4.8% of the intensity of each fragment of interest, and each of the mass spectral peaks was corrected for this (Line C). Thus, the peak at $m/e$ 161 includes a contribution from this interfering fragment, with 4 atoms of $^{18}O$/molecule. This contribution (0.048 x 19.0%) is subtracted from 161 and added to 163 to give the true percentage with 4 atoms of $^{18}O$/molecule.

The other peaks are treated alike. Correction for incomplete solvent isotope enrichment (measured as described above) is shown in Line D, which represents the distribution if the isotopic enrichment had been 100%. Control samples with no fibers showed a small amount of spontaneous ATP hydrolysis in solution (typically 1% of that produced by the fiber), as well as the 0.2% natural abundance of $^{18}O$. Corrections are made for these in Line E, which is then normalized to give Line F.

The final sets of data (as in Line E), after all corrections had been made, were close to the distributions in the raw mass spectral data, except for fragments $m/e$ 161 (3 solvent oxygens). The changes at this mass were due almost entirely to the correction for incomplete $^{18}O$ enrichment of water. The sensitivity of $m/e$ 161 to the $^{18}O$ enrichment of the water was a cause for concern since the enrichment appeared to drop up to 2% during the course of an experiment. A further problem was that the method for measuring the $^{18}O$ enrichment (see above) inherently gave a lower limit. However, these concerns were offset by high reproducibility in the relative compositional similarity of fragments in repeated experiments.

The results described below show that no single distribution fits the data well. Data could be fitted by considering that ATP hydrolysis occurs through two different pathways each with a distinct value of $R$ ($= k_{cA}/k_{cB}$). For such a combination of pathways, each value of $R$ and the relative fluxes between the two pathways were adjusted to minimize $\Delta^2$, the least squares summation of the differences between the observed (as in Table II) and theoretical peak heights (expressed as percentages). This procedure was also used to investigate the compatibility of the data with more restrictive models of the pathway of ATP hydrolysis (Table III) by determining whether $\Delta^2$ falls outside that expected from experimental errors.

The experimental error was calculated for each sample as follows. The sum of the four peaks areas (157–163) for each injection was normalized to total 100% to provide direct comparison with the final set of data, such as Line F in Table I. From the data of three injections, the standard errors from the mean were determined for these four normalized peak areas. The largest standard error (S.E.) was used to calculate the experimental variation in terms of the sum of the squares of errors, 4 (S.E.). So a theoretical pathway, or combination of pathways, is compatible with the experimental data if $\Delta^2 < 4$ (S.E.).

However, this test should only be considered as a guideline because of potential systematic errors arising from incomplete $^{18}O$ enrichment of water and from its measurement.

### RESULTS AND DISCUSSION

After ATP was hydrolyzed by fibers in $^{18}O$-enriched water, mass spectral analysis of the product P, showed the presence of $^{18}O$ due to oxygen exchange. Following corrections for incomplete isotopic enrichment of the solvent and spontaneous ATP hydrolysis, as described under "Experimental Procedures," the distributions of $^{18}O$-labeled P, species were obtained and are shown in Table II. One solvent oxygen is incorporated into each P, molecule, even if there is no exchange. All P, containing more than one solvent oxygen has undergone oxygen exchange.

In the experiments with relaxed fibers the major species of P, contained 4 solvent oxygens indicating that there was extensive oxygen exchange. However, as quantified in Footnote c of Table II, the observed distribution of P, species did not correspond to a single pathway of ATP hydrolysis. This was the case in each experiment and at least two pathways were required to achieve good correspondence between the theoretical P, distributions expected and the data (Fig. 1 and Table II). The relaxed fiber distributions show a striking resemblance to those obtained with myosin (Shukla and Levy, 1977; Sleep et al., 1980). The major pathway has a high level of oxygen exchange ($R < 0.02$) as expected for the myosin ATPase without interaction with actin. The origin of P, formed with little exchange ($R \approx 2$) is not clear. For the isolated protein, it is probably due to spontaneous modification of myosin (such as thiol oxidation) or contaminating ATPases (Shukla et al., 1983), and may also be true of the relaxed fibers.

Alternatively the low exchange fraction of P, may have resulted from ATP hydrolysis by a small population of the myosin heads interacting with actin in the thin filaments in spite of the low concentration of free Ca$^{2+}$. To test this possibility two experiments were performed with bundles of 7 and 8 fibers stretched to a length at which overlap of the thick and thin filaments was zero (beyond a sarcomere length of 3.6 $\mu$m). The overall ATPase activities and oxygen exchange patterns were the same (within experimental error) to those obtained in the experiments at full overlap. Thus, the low exchange pathway observed with relaxed fibers was not dependent on filament overlap.

Compared with relaxed fibers, the Ca$^{2+}$-activated fibers hydrolyzed ATP rapidly and with relatively little oxygen exchange. The major P, species contained only one solvent oxygen (Fig. 1). Like relaxed fibers, a minimum of two pathways of ATP hydrolysis was required to accommodate the distribution of oxygen isotope observed in the product P,.

Ca$^{2+}$-regulated ATPase activation was approximately 30-fold (Table II). Thus, any contaminant ATPase contributing to the product P, would need to be Ca$^{2+}$-sensitive. Various controls were carried out to test for the maximum amount of P, that could have been formed in the Ca$^{2+}$-activated experiments other than by actomyosin catalysis. ATPase activity and oxygen exchange characteristics were measured when fibers were stretched beyond filament overlap in a solution.
who showed that 32P exchange occurred between ATP and protein-bound ADP. This implies that oxygen exchange characteristic of actomyosin ATPase activity within the fibers. and distribution of oxygen isotopes in the Pi formed was characteristic of medium Pi during actosubfragment 1 ATPase activity. This is suggested by the work of Sleep and Hutton (1980) and is probably due to reformation of ATP from Pi and exchange is probably due to reformation of ATP from Pi and protein-bound ADP. This implies that oxygen exchange beyond overlap was indistinguishable from that found in experiments at full overlap. Furthermore, the distribution of oxygen isotopes in Pi formed from ATP hydrolysis catalyzed by sarcomeres stretched beyond overlap was indistinguishable from that found in experiments at full overlap.

A second test for other sources of ATPase activity was to hydrolyze ATP in fibers stretched beyond overlap in the presence of 0.5 mM quercetin and 1 μg·ml⁻¹ oligomycin to minimize contaminating sarcoplasmic reticulum Ca²⁺-ATPase and mitochondrial ATPase activities (Penefsky, 1979; Shoshan and MacLennan, 1981). In this case the ATPase activity was 11% of that at full overlap, so that a maximum of 9% of the activity observed at full overlap could have been due to these two sources of ATPase activity.

The principal conclusions from these control experiments are that most or all of the activity observed in Experiments 3 and 4 (Table II) was due to actomyosin ATPase and that the distribution of oxygen isotopes in the Pi formed was characteristic of actomyosin ATPase activity within the fibers. Another possible source of oxygen exchange in activated fibers is suggested by the work of Sleep and Futton (1980) who showed that ³²P exchange occurred between ATP and medium Pi during actosubfragment 1 ATPase activity. This exchange is probably due to reformation of ATP from Pi and protein-bound ADP. This implies that oxygen exchange between water and Pi in the medium may be possible during ATPase activity. We checked for any effect of this oxygen exchange reaction on our results by including 2 mM unlabeled Pi at the start of an experiment with an activated fiber. No significant difference was found between the distribution of oxygen isotopes in Pi in that experiment and those of Experiments 3 and 4 (Table II).
We therefore conclude that the general pattern of oxygen exchange accompanying ATP hydrolysis in Ca²⁺-activated fibers is that expected from a scheme in which the life-time of a myosin- or actomyosin-products complex is decreased compared to that at low Ca²⁺ concentration. The properties of this Ca²⁺-dependent ATPase are thus similar to that of unregulated actomyosin in solution. Both the extent of exchange and the ATPase activity are similar to those found with actomyosin (ATPase activity = 2.8 s⁻¹ at pH 7 and 25 °C) (Sleep et al., 1980). In contrast, actosubfragment 1 has a larger ATPase activity (= 40 s⁻¹, at pH 8 and 25 °C, Weeds and Taylor, 1975) and much less oxygen exchange is associated with its ATPase (Sleep and Boyer, 1978).

In principle k₋2 can be estimated from turnover rates of the ATPase and the calculated values of R (Table II). In the relaxed fiber experiments we noted that the ATPase activities of the fractions with high R values may have been derived from extraneous ATPase activity. In that case the turnover rates due to myosin alone in Experiments 1 and 2 were proportionately lower with values of 0.10 s⁻¹ and 0.043 s⁻¹. Since the turnover rate for myosin is determined by k₊2, and k₋2 = k₋₁/R it follows that k₋₂ ≈ 9 and 12 s⁻¹, respectively. These values are close to that estimated for k₋₂ from studies of myosin subfragment 1 in solution (Webb and Trentham, 1981).

If the same process is performed on the data from activated fibers (Table II), k₊₂ ≈ 1 s⁻¹ for the major pathway and ∼ 10 s⁻¹ for the minor one. This might suggest that k₊₂ is reduced relative to its value in the relaxed fiber. However, a more likely hypothesis is that on the major pathway k₊₂ is unaltered by Ca²⁺ activation, and that k₋₂, the rate constant for Pᵢ release, is an order of magnitude greater than the overall ATPase rate. In the case of activated fibers the measured ATPase rate may not reflect the real, per head rate, since not all the heads may be involved in ATP hydrolysis. This might be due to incorrect positioning for interaction with the thin filament or due to partial inactivation during the 2-3-h incubation time. These factors would cause the measured rate to be less than the real rate, although they are unlikely to account for the order of magnitude difference between k₊₂ and the ATPase rate. The conclusion that then follows is that the rate constant of a step subsequent to Pᵢ release is markedly reduced in a fiber held isometrically. However, it is not clear whether the mechanical state is controlling the oxygen-exchange kinetics because of the similar oxygen exchange patterns in activated fibers and actomyosin (Sleep et al., 1980).

As shown in Table III the pattern of oxygen exchange during Ca²⁺-activated activity is not compatible with a single pathway of ATPase hydrolysis. A number of mechanisms have been proposed to explain how such a pattern might arise with actomyosin. Sleep et al. (1980), after considering several possibilities, have suggested that it is most likely that the complex exchange pattern derives from geometric constraints within the filament lattice causing varying degrees of accessibility between the myosin heads and actin. Mideflord (1981) favored the idea that the direct hydrolysis pathway, in which ATP hydrolysis occurs on associated actomyosin, contributes to the exchange pattern, although Hackney (1982) has pointed out that, at least in the case of the acto heavy meromyosin ATPase, this cannot be the sole explanation. Shukla et al. (1983) consider that there are two pathways of ATP hydrolysis with equal flux derived from distinct properties of the two heads of myosin during actomyosin ATPase activity. We tested this last possibility by calculating the minimum value of Δ₂ obtained with the constraints of the mechanism of Shukla et al. (1983). Table III shows in one case the resulting value of Δ₂ is relatively large, but our data do not rule out their hypothesis.

In conclusion it has been shown that measurement of oxygen exchange within relaxed and active fibers is possible. The data are compatible with a mechanism of ATP hydrolysis that incorporates a reversible cleavage step followed by slow release of Pᵢ in relaxed fibers and an enhanced rate of release in activated fibers. The enhanced rate of release is sufficient to suggest that in an activated fiber held isometrically, Pᵢ release may precede the rate-limiting step. The overall pattern of exchange in fibers parallel those accompanying myosin and actomyosin ATPase activity in solution and suggest intrinsic similarity between the mechanisms of ATP hydrolysis catalyzed by fibers and isolated contractile proteins.

REFERENCES

Abbott, R. H., and Steiger, G. J. (1977) J. Physiol. (Lond.) 266, 13-42

Bartlett, P. W., Cox, R. N., Kawai, M., and Robinson, T. (1982) J. Gen. Physiol. 79, 997-1016

Cooke, R., and Bialek, W. (1979) Biophys. J. 28, 241-258

Ford, L. E., Huxley, A. F., and Simmons, R. M. (1977) J. Physiol. (Lond.) 269, 441-515

Goldman, Y. E., Hibberd, M. G., and Trentham, D. R. (1984a) J. Physiol. (Lond.) 354, 577-604

Goldman, Y. E., Hibberd, M. G., and Trentham, D. R. (1984b) J. Physiol. (Lond.) 354, 605-624

Goldman, Y. E., and Simmons, R. M. (1984) J. Physiol. (Lond.) 360, 497-518

Granath, I. (1976) Top. Phosphorus Chem. 8, 41-98

Hackney, D. D. (1982) J. Biol. Chem. 257, 9494-9500

Hackett, D. D., Stempel, K. E., and Boyer, P. D. (1980) Methods Enzymol. 64, 60-88

Huxley, A. F. (1975) Prog. Biophys. Biophys. Chem. 7, 255-318

Huxley, A. F., and Niedergerke, R. (1984) Nature 213, 971-973

Huxley, H. E. (1966) Science 154, 1356-1366

Huxley, H. E., and Hanson, J. (1954) Nature 173, 973-976

Koshland, D. E., and Clarke, E. (1953) J. Biol. Chem. 206, 917-924

Levy, H. M., and Koshland, D. E. (1959) J. Biol. Chem. 234, 1102-1112

Lowey, S., Slattery, H. S., Weeds, A. G., and Baker, H. (1969) J. Mol. Biol. 42, 1-29

Lynn, R. W., and Taylor, E. W. (1971) Biochemistry 10, 4617-4624

Mideflord, C. F. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 2067-2071

Moesciu, D. G. (1976) Nature 262, 610-613

Peneskis, H. S. (1979) The Enzymes 55, 297-303

Shoshan, V., and MacLennan, D. H. (1981) J. Biol. Chem. 256, 887-892

Shukla, K. K., and Levy, H. M. (1977) Biochimica et Biophysica Acta 162, 132-136

Shukla, K. K., Levy, H. M., Ramirez, F., Marecek, J. F., McKeever, B., and Margossian, S. S. (1983) Biochemistry 22, 4822-4830

Sleep, J. A., and Boyer, P. D. (1979) Biochemistry 15, 5417-5422

Sleep, J. A., and Hutton, R. L. (1980) Biochemistry 19, 1276-1283

Sleep, J. A., Hackney, D. D., and Boyer, P. D. (1980) J. Biol. Chem. 255, 4094-4099

Webb, M. R., and Trentham, D. R. (1981) J. Biol. Chem. 256, 10910-10916

Webb, M. R., and Trentham, D. R. (1983) in Handbook of Physiology (Peachey, L. D., Adrian, R. H., and Geiger, S. R., eds) pp. 237-255, American Physiological Society, Washington, D.C.

Weeds, A. G., and Taylor, R. S. (1975) Nature 257, 54-56