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Phosphorus-31 Nuclear Magnetic Resonance Studies of Single Muscle Cells Isolated from Barnacle Depressor Muscle†

George R. Dubyak* and Antonio Scarpa

ABSTRACT: ^{31}P NMR spectra (145.7 MHz) were recorded from single muscle cells isolated from the depressor muscle of the barnacle *Balanus nubilis*. The single cells, mounted in a small (2.2-mm) diameter solenoidal receiver coil, were scanned by using 45° pulses with delay times ranging from 1 to 10 s; 400 scans (10–15-min total accumulation time) were sufficient to obtain spectra with good signal/noise ratios. Spectra obtained within 30 min after dissection reveal that phosphoarginine and ATP are the only phosphometabolites present in appreciable (>1 mM) concentration in these cells under resting conditions. As the postdissection time is increased, the phosphoarginine content decreases 6–8%/h. This depletion is matched by concomitant increases in the concentrations of sugar phosphate esters and inorganic phosphate; ATP is maintained at constant levels during these shifts in phosphometabolite pools. The rates of sugar phosphate and inorganic phosphate accumulation are increased by exposing the cell to iodoacetate and cyanide (cyanide alone has no effect). Quantitative analysis of both the in vivo NMR data and the in vitro chemical analysis of single-cell extracts indicates that these cells contain about 60 mM phosphoarginine and 7 mM ATP. The in vivo data suggest that sugar phosphate and inorganic phosphate contents are within the 0.1–1.0 mM range. While the ADP content of the cell extracts is approximately 1 mM, the near-equivalence of the three ATP resonance signals observed in situ suggests that the sarcoplasmic content of ADP is <0.2 mM. Furthermore, if it is assumed that arginine kinase operates near equilibrium in these cells, a sarcoplasmic [ADP] of about 0.04 mM can be calculated.

^{31}P NMR in increasingly being used as a noninvasive probe of phosphometabolite content and turnover and of intracellular ionic conditions in intact muscle; such studies have been performed by using skeletal (Dawson et al., 1977; Gadian et al., 1981; Meyer et al., 1982), cardiac (Ingwall, 1982; Wu et al., 1981), invertebrate (Barrow et al., 1980), and smooth (Dillon et al., 1983) muscle types. The NMR spectra of such multicellular muscle preparations result from an averaging of the phosphometabolite levels or ionic parameters within the various component cells. Important differences between cells

† From the Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104. *Received February 14, 1983.* This work was supported by National Institutes of Health Grant HL-15835 to the Pennsylvania Muscle Institute and in part by a grant from R. J. Reynolds Industries.

0006-2960/83/0422-3531$01.50/0

may be obscured in this averaging process, particularly if the tissue contains a number of cell types. While this problem is substantially reduced when muscles containing relatively homogeneous populations of cells are studied, care must be taken to provide a uniform supply of oxygen and other substrates so as to ensure metabolic homogeneity among the individual cells. Such problems relating to heterogeneity of cell type and/or metabolic state would de facto be obviated in a single muscle cell preparation which possesses sufficient mass for analysis by current ^{31}P NMR techniques.

A suitable candidate for such investigations is the giant muscle cell which may be isolated from the depressor muscles of the barnacle *Balanus nubilis* (Hoyle & Smyth, 1963). These single cells are 1–2 mm in diameter, up to 60 mm in length, and 20–80 mg in wet weight. The cells have been extensively used in investigations aimed at the direct measurement of intracellular ionic parameters including nonsolvent water space (Gayten & Elliott, 1980), monovalent cation and anion activities (Hinke et al., 1973), pH (Boron, 1979), free Ca^{2+} (Ashley & Ridgway, 1970; Dubyak & Scarpa, 1982), and free Mg^{2+} (Brinley et al., 1977). The large size of these cells permits their cannulation, and the cannulated end of the cell provides a ready access to the cell interior. Microsyringes or ion-specific electrodes can be longitudinally inserted into the cell for the introduction of precise quantities of normally membrane-impermeable compounds or the measurement of intracellular ionic activities. Thus, critical intracellular ionic parameters (e.g., pH and pMg) which modify the chemical shifts of most phosphometabolites can be precisely defined and even manipulated in these cells.

Using a specially designed receiver coil, we have been able to record ^{31}P NMR spectra with good signal/noise ratios (S/N ratios) from these single muscle cells in relatively short periods of data acquisition. These single-cell spectra are identical with those obtained by using the whole depressor muscles from which the single cells are isolated. These in vivo experiments have been supplemented by analytical measurements on extracts of single cells so as to permit absolute quantitation of the major intracellular phosphometabolites. The results indicate that this single-cell system should prove useful in addressing a number of problems germane to the application of ^{31}P NMR as a quantitative probe of muscle metabolism.

Materials and Methods

Muscle Preparation. The tissues from which the single cells are isolated are the rostral and lateral depressor muscles of the barnacle *Balanus nubilis*. These muscles (1–2-g wet weight) are composed of bundles of 60–70 individual cells; their morphology has been extensively described by Hoyle & Smyth (1963). Prior to planned experiments, a bundle is bathed for at least 90 min in artificial seawater (ASW) containing less than 10 μM Ca^{2+}. This effectively prevents activation of the inward Ca^{2+} currents which initiate excitation–contraction coupling in the cells.

Measurements of ^{31}P NMR Spectra Using Whole Barnacle Depressor Muscles. Bundles of approximately 20 single cells (400–800-mg wet weight) were separated from the intact muscle by cutting the fibers through their multitendonous shell insertion at one end and through single tendons at the other end. The bundle was then slid, single tendon end first, into a 10-mm diameter NMR sample tube. When bundles containing at least 20 cells greater than 3 cm in length were used, the bottom portion of the tube was almost completely occupied by the tissue mass. The bundle was then washed 3 times (2 min each) with 2 volumes of low Ca/ASW containing 15–20% D_2O for signal locking. It must be emphasized that in all

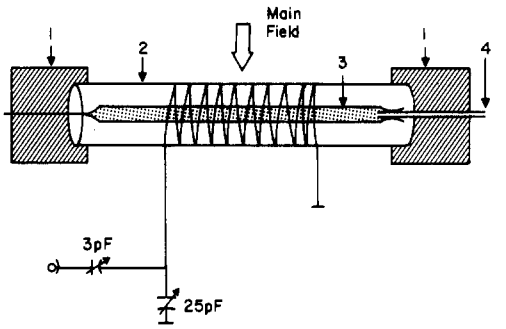

FIGURE 1: Schematic diagram (not to scale) of probe assembly used in single-cell NMR experiments. A single muscle cell (3) is cannulated at one end with an 800-μm glass capillary (4). The cell is contained within a sample tube (2) (dimensions: 2.2-mm outside diameter, 0.2-mm wall thickness, 25-mm length) filled with artificial seawater. The sample tube is sealed at both ends with silicone rubber plugs (1). The sample tube snugly fits into a 10-turn receiver coil (10-mm length) made of enameled copper wire.

NMR experiments described herein (with muscle bundles or single cells), the tissue or cell was *not* superfused with fresh medium and the medium is *not* continuously oxygenated during acquisition of the NMR spectra. ^{31}P NMR spectra were obtained at 145.7 MHz by using a Bruker WH360 spectrometer. Spectra were routinely accumulated by using 90° (23-μs) radio-frequency pulses, a sweep width of 10000 Hz, 1–10-Hz line broadening, and delay times ranging from 0.1 to 10 s. All experiments were performed at room temperature (18–21 °C). All chemical shifts are expressed relative to the usual standard of 85% (by volume) inorganic orthophosphate.

Measurement of ^{31}P NMR Spectra Using Single Barnacle Muscle Cells. Single muscle cells were isolated and cannulated by using previously described techniques (Dubyak & Scarpa, 1982). The entire isolation and cannulation procedure could be completed within 10 min. For NMR studies, 25-mm lengths of single fiber with diameters greater than 900 μm were routinely used; the wet weight of such preparations was 15–20 mg. Immediately after cannulation, the fiber was mounted in a specially designed probe assembly (Figure 1). The receiver coil was tuned by using the variable capacitors before and after the sample capillary was mounted. After the capillary holder was mounted, acetone was carefully dripped onto the coil and then evaporated by holding the coil in a stream of dry air. This latter procedure removed any moisture (which may accumulate during the mounting of the capillary) adhering to the coil. The entire assembly was mounted in an aluminum tube and inserted into the probe chamber of the 360-MHz magnet. The entire mounting procedure required approximately 15 min. The spectrometer operating parameters for these single-cell experiments were identical with those used for studies of muscle bundles with the exception that the pulse duration was reduced to 5 μs (45°); this latter duration was found to produce spectra with optimal S/N ratios.

Analytical Techniques. Metabolites were extracted from single muscle cells by using a modification of a previously described acid/alcohol extraction protocol (Schulz et al., 1967). A single cell was weighed, frozen in liquid nitrogen, and then homogenized in 1.0 mL of 60% ethanol/1 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0) at −15 °C. The suspension was slowly diluted to 4.0 mL with 0.4 N perchloric acid, incubated at 0 °C for 10 min, and then centrifuged at 5000*g* for 5 min. The supernatant was neutralized with 3 N KOH, and the precipitated perchlorate was pelleted by centrifugation. Phosphoarginine and nucleotide levels in the extracts were determined by using reversed-phase high-pressure

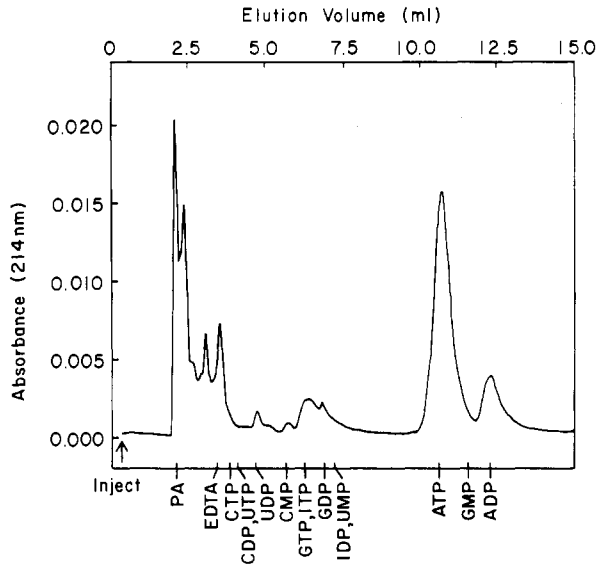

FIGURE 2: HPLC elution profile of an acid/alcohol extract of a single barnacle muscle cell. Details of the cell extraction and chromatographic protocol are provided under Materials and Methods. At zero time, a 10-μL aliquot of the cell extract was injected. Also shown are the elution volumes observed for calibrating samples of phosphoarginine, EDTA, and purine/pyrimidine nucleotides.

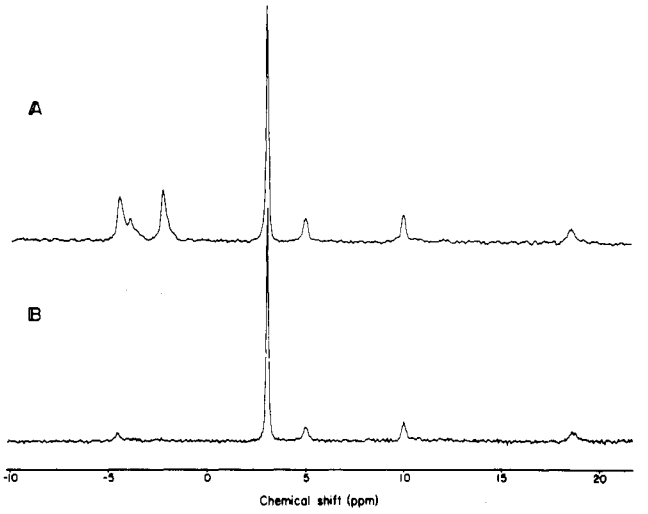

FIGURE 3: ^{31}P NMR spectra of a fresh barnacle depressor muscle. A bundle of about 20 single cells (500-mg wet weight) was immersed in Ca-free seawater contained in a 10-mm diameter sample tube. Five hundred 90° radio-frequency pulses (23-μs duration) were applied with 1.2-s intervals between each pulse acquisition cycle (0.82-s acquisition time). The signal-averaged frequency domain spectra were obtained in about 20 min. Spectrum B was obtained immediately after addition of the muscle, while spectrum A was obtained after incubation of the isolated muscle for 7 h.

liquid chromatography employing a C18 Bondapak 5-μm cartridge (Waters Associates). Samples were isocratically eluted with a mobile phase of 0.3 M ammonium phosphate (pH 4.2) pumped at 2–3 mL/min. This chromatographic system facilitates clean separation of phosphoarginine and ATP from the mono-, di-, and triphosphate esters of guanosine, inosine, cytidine, and uridine. Since the elution volume of ADP is partially overlapped by that of GMP, precise quantitation of ADP can be complicated by the presence of significant GMP levels. However, a representative chromatogram of a single muscle cell extract (Figure 2) demonstrates that phosphoarginine, ATP, and ADP constitute the major proportion of extracted phosphometabolites. Inorganic phosphate content was measured by using a coupled phosphorylase/phosphoglucomutase/glucose-6-phosphate dehydrogenase assay system and monitoring the production of reduced NADPH (Schulz et al., 1967). L-Arginine was enzymatically measured by using an octopine dehydrogenase based assay system (Gaede & Grieshaber, 1975).

Results

^{31}P NMR Spectra of Whole Barnacle Depressor Muscles. This research was directed toward measurement of phosphometabolite levels within single barnacle muscle cells. Studies using the muscle bundles from which these single cells are isolated were, however, prerequisite in order to characterize the ^{31}P NMR spectra of the cells prior to the fairly extensive (and possibly damaging) manipulations involved in preparation of the single cells for NMR experiments. Figure 3B shows a representative spectrum obtained within 15 min after dissection of a rostral depressor muscle comprising about 20 single cells. In general, the signal averaging of 100 scans (approximately 5-min accumulation using a 1.2-s delay) produced spectra with excellent signal/noise ratios. Invariably, the major signal at 3.5 ppm and three smaller signals at 4.9, 9.9, and 19 ppm were the only readily apparent peaks observed in freshly isolated muscle. The integrated areas under each of the smaller signals were equivalent and usually 7–10 times smaller than the major resonance. Given the cytosolic pH of 7.3 (Boron, 1979) and the pMg of 2.2 (Brinley et al., 1977) which characterize these cells, the smaller peaks at 4.9 and 9.9 ppm may respectively represent γ- and α-phosphates of nucleotide triphosphates and/or the β- and α-phosphates of nucleotide diphosphates; the peak at 19 ppm is characteristic of the β-phosphate of nucleotide triphosphates. Since quantitative analysis (Figure 2) shows that ATP constitutes by far the major proportion of extracted nucleotides, the signals at 4.9, 9.9, and 19 ppm emanate for the most part from cellular ATP pools. The major peak at 3.5 ppm may be identified as phosphoarginine, the phosphogen in most invertebrate muscle types (Ennor & Morrison, 1958). No significant signal was observed at approximately −2.6 ppm, the location of inorganic phosphate (P_i) at pH 7.3. Likewise, only a very small peak could be discerned at −5 ppm, the usual location of sugar esters at this pH (Barrow et al., 1980). As the postdissection time increased (Figure 3A), the relative intensities of the signals at −5 and −2.6 ppm steadily increased, that at 3.5 ppm (phosphoarginine) decreased, and those at 4.9, 9.9, and 19 ppm remained constant. In Figure 4, the relative levels of the various phosphometabolites were determined from sequential spectra of a given muscle bundle and plotted as a function of postdissection time. In the experiment depicted in Figure 4A, the bundle was immersed in normal Ca-free ASW, while in the second experiment (Figure 4B) the bundle was immersed in Ca-free ASW containing 2 mM cyanide.

^{31}P NMR Spectra of Single Barnacle Muscle Cells. Using the probe assembly illustrated in Figure 1, we performed a similar series of experiments with cannulated single muscle cells. Figure 5 shows a sequence of ^{31}P NMR spectra, each of which was obtained by signal averaging 800 45° pulses with a delay of 1.2 s between pulses (total acquisition time 33 min). The observed shift positions of major resonance signals were very similar to those observed with whole depressor muscles (Figure 3), i.e., sugar phosphates at −5 ppm, P_i at −2.5 ppm, phosphoarginine at 3.5 ppm, γ-P of ATP at 4.9 ppm, α-P of ATP at 9.9 ppm, and β-P of ATP at approximately 18.5 ppm. Single-cell spectra obtained within 60 min after cannulation and mounting in the probe were characterized by very low levels of sugar phosphates and inorganic phosphates. With prolonged incubation in the noncirculating pool of saline, the

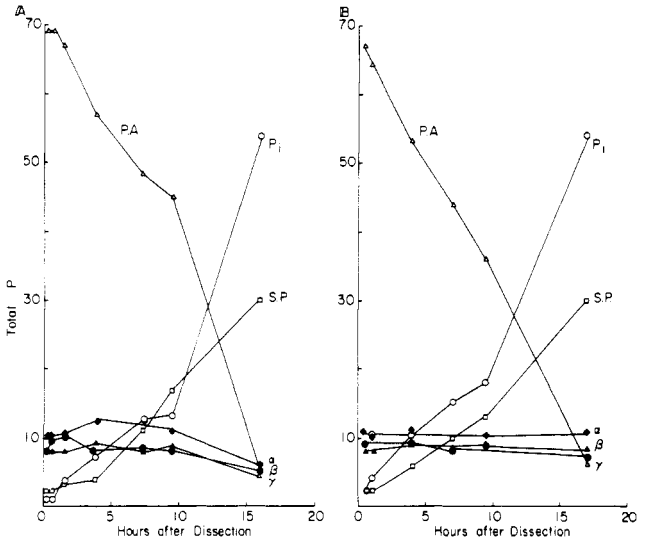

FIGURE 4: Relative phosphometabolite levels in barnacle depressor muscle at various times after isolation. Sequential spectra of barnacle muscle maintained in normal Ca-free seawater (A) and Ca-free seawater containing 2 mM cyanide were used for calculation of relative phosphometabolite levels. Each point represents the area of a given resonance divided by the area under all major resonance signals. Since each barnacle muscle was maintained in the same pool of saline throughout the experiments, the total ^{31}P content remained constant. (△) Phosphoarginine; (○) inorganic phosphate; (□) sugar phosphates; (♦, ●, and ▲) α-, β-, and γ-phosphates of ATP.

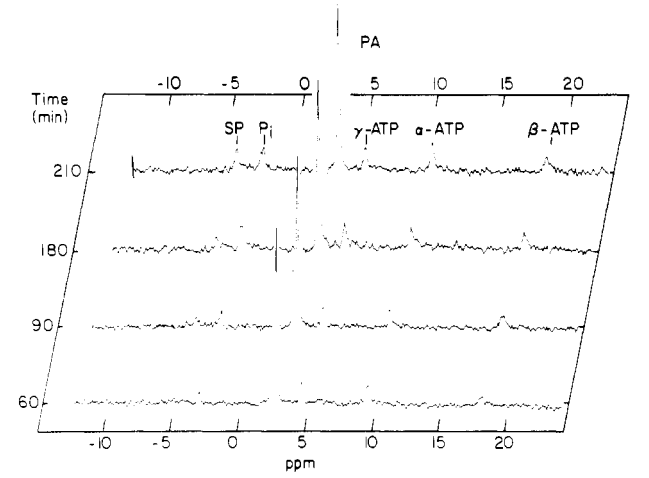

FIGURE 5: ^{31}P NMR spectra of a single barnacle muscle cell at various times after dissection. A single (22-mg wet weight) muscle cell was mounted in the assembly illustrated in Figure 1. Eight hundred 45° (5-μs duration) radio-frequency pulses were applied with a 1.25-s delay between each pulse acquisition cycle. Each signal-averaged spectrum was thus obtained in 26 min. Zero time represents the point at which the cell was placed in the sample capillary.

concentrations of both these species gradually increased. When a single cell was incubated in seawater containing 2 mM iodoacetate and 2 mM cyanide, these changes in phosphometabolite levels were greatly accelerated (Figure 6). After only 30 min of incubation, the apparent concentrations of both P_i and sugar phosphate exceeded that of ATP. After 60 min, sugar phosphate was the predominant ^{31}P-containing species, although ATP was held at near-control levels. After 2 h, all the phosphoarginine and ATP were consumed, and cellular phosphorus was pooled largely in the form of sugar phosphates. These time-dependent shifts in the phosphometabolite profiles of control and poisoned single cells are illustrated in Figure 7. It should be pointed out that in Figure 6 the first control spectrum and the three poisoned cell spectra were each obtained after only 13 min (400 scans) of data accumulation.

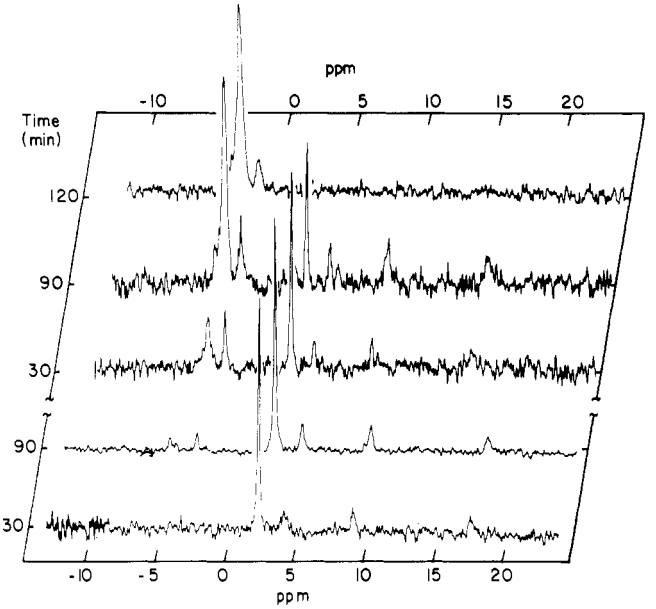

FIGURE 6: ^{31}P NMR spectra of a single barnacle muscle cell incubated in the presence of iodoacetate and cyanide. Spectra were obtained as described in Figure 5. Each spectrum (with the exception of the control 90-min spectrum) was accumulated over 400 scans (13-min total acquisition time). The first 30- and 90-min spectra were obtained from a single control muscle cell incubated in normal Ca-free seawater. A second cell from the same muscle bundle was then isolated and placed in Ca-free seawater containing 2 mM iodoacetate and 2 mM cyanide; spectra were subsequently recorded at 30, 90, and 120 min after the cell was placed in the latter medium.

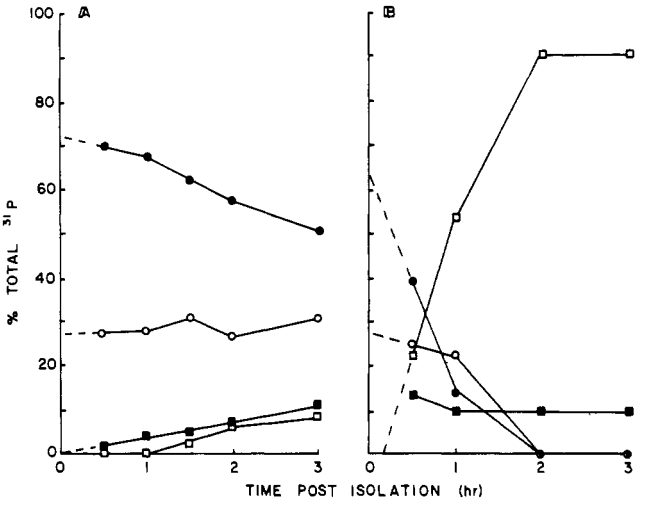

FIGURE 7: Relative phosphometabolite levels in single barnacle muscle cells at various times after incubation in the presence and absence of metabolic inhibition. Relative phosphometabolite levels within single muscle cells were calculated as previously described from the sequential spectra illustrated in Figures 5 and 6. (●) Phosphoarginine; (○) sum of α-, β-, and γ-phosphates from ATP; (■) inorganic phosphate; (□) sugar phosphates.

Quantitation of Phosphometabolites within Single Muscle Cells. To supplement the data obtained from ^{31}P NMR studies, we prepared extracts of single cells for quantitative analysis of phosphoarginine, L-arginine, inorganic phosphate, and adenosine phosphates. The apparent intracellular contents of these species are listed in Table I.

Discussion

The ^{31}P NMR spectra of both barnacle depressor muscle (Figure 3) and single muscle cells derived from this tissue (Figure 5) were quite simple, with phosphogen (phosphoarginine) and ATP contributing the only appreciable resonance

Table I: Metabolite Contents in Extracts of Single Muscle Cells[a]

metabolite	content (mmol/kg wet weight)
phosphoarginine	42.5 ± 1.5 (n = 12)
L-arginine	3.8 ± 0.4 (n = 5)
ATP	4.2 ± 0.2 (n = 12)
ADP	1.1 ± 0.2 (n = 12)
inorganic phosphate	2.2 ± 0.5 (n = 5)

[a] Single fibers were extracted as described under Materials and Methods. Phosphoarginine, ATP, and ADP contents were determined by reversed-phase HPLC, while L-arginine and inorganic phosphate were quantified by using enzyme assays. Values represent the mean ± the standard error of data obtained from separate single fibers; n refers to the number of single fibers analyzed.

signals (Figure 2). In this regard, the barnacle muscle spectra are qualitatively most similar to those obtained from resting fast skeletal muscle (Dawson et al., 1977; Gadian et al., 1981). In contrast, the spectra from resting slow skeletal (Meyer et al., 1982), smooth (Dillon et al., 1983), cardiac (Ingwall, 1982; Wu et al., 1981), and molluscan (Barrow et al., 1980) muscles variously show considerable levels of inorganic phosphate, sugar phosphates, or pyridine nucleotides. Sugar phosphates and inorganic phosphate appeared in the whole muscle and single-cell spectra only after prolonged incubation in a noncirculating pool of extracellular saline containing no metabolite substrates (Figures 3–5 and 7). Concomitant with these latter changes was a gradual decrease (6–8%/h) in the apparent concentration of phosphoarginine; the relative ATP content was unchanged throughout. For resting whole barnacle muscle (Figure 4B) and single muscle cells (data not shown), inclusion of 2 mM cyanide in the extracellular fluid did not appreciably alter the rates of phosphoarginine depletion or inorganic phosphate/sugar phosphate accumulation. This would indicate that oxidative phosphorylation does not significantly contribute to basal ATP synthesis in resting barnacle muscle. Such a supposition is consistent with the sparse mitochondrial content of these muscle cells (Hoyle et al., 1973). In contrast, exposure of single muscle cells to 2 mM iodoacetate plus 2 mM cyanide resulted in a rapid and massive accumulation of sugar phosphates which coincided with absolute depletion, first of phosphoarginine and then of ATP (Figures 6 and 7B). A similar effect of alkylating agents was reported by Barrow et al. (1980) in a ^{31}P NMR study of invertebrate muscle isolated from the mollusc *Tapes watlingi*. The result is consistent with potent inhibition by iodoacetate and iodoacetamide of various glycolytic enzymes. Although these agents react with essential thiol grops of arginine kinase (Virden & Watts, 1966), the alkylation and inactivation of this phosphotransferase are considerably retarded by the presence of various intracellular substrates, e.g., arginine and MgATP. Furthermore, since the cell was incubated in substrate-free saline, the massive accumulation of sugar phosphates indicated the presence of substantial stores of endogenous substrate. Hoyle et al. (1973) noted that conspicuously dense populations of glycogen particles characterize the sarcoplasm of these cells.

The outstanding quantitative characteristics of the spectra from whole barnacle muscle and single muscle cells are the following: (1) the very low inorganic phosphate content; (2) the high phosphoarginine content; (3) the equivalence of the integrated areas under three peaks presumed to represent ATP. Data obtained within 30 min after placing whole muscles or single cells within the magnet (Figures 4A and 7A) consistently showed that the area under the inorganic phosphate peak was 10–15% of that under any of the three ATP resonance signals. This was observed in experiments wherein the delay between successive pulse acquisition cycles ranged from 1.2 to 10 s, thus indicating that saturation of inorganic phosphate relaxation parameters did not account for the low P_i/ATP ratios. Similar analysis of relative peak areas revealed that initial phosphoarginine/ATP ratios were 7.6 ± 0.7 (n = 7) in whole barnacle muscles and 7.8 ± 0.6 (n = 4) in single muscle cells. In all spectra, the areas under the 4.9 and 19 ppm peaks varied by less than 5–8%; this implies that the sarcoplasmic ADP concentration was at least 1 order of magnitude lower than that of ATP. Thus, ^{31}P NMR measurements indicate that phosphoarginine, ATP, inorganic phosphate, and ADP are present in barnacle sarcoplasm at approximate proportions (ATP = 1) of 8:1:0.1:0.05.

These relative proportions estimated from in vivo experiments may be compared with absolute metabolite levels measured in acid/alcohol extracts of single barnacle muscle cells (Table I). In these extracts, phosphoarginine, ATP, inorganic phosphate, and ADP were present in relative proportions (ATP = 1) of 10:1:0.5:0.25. Thus, while the phosphoarginine/ATP ratios determined in vivo and in vitro differ by only 20%, the respective ATP/P_i and ATP/ADP ratios differ by factors of 5. These latter discrepancies most likely reflect (1) effects of artifactual phosphometabolite hydrolysis (enzymatic and nonenzymatic) during extraction of the muscle and (2) intracellular compartmentation of phosphometabolites. For example, of the 1.1 mmol of ADP/kg wet weight extracted, at least 0.4 mmol/kg represents ADP bound to F-actin (Seraydarian et al., 1962). Furthermore, since unattached cross bridges retain the products of ATP hydrolysis, approximately 0.2 mmol of ADP and P_i/kg wet weight will be bound to myosin heads.

Consolidation of the in vivo NMR data and the in vitro analytical results allows us to estimate the following metabolite concentrations within barnacle muscle sarcoplasm, assuming 70% of the total wet weight represents intercellular water (Clark & Hinke, 1981): 60 mM phosphoarginine, 5.5 mM L-arginine, 6.7 mM ATP, <0.2 mM ADP, and <0.7 mM inorganic phosphate. Brinley et al. (1977) have shown that most of the ATP exists as MgATP. If it is assumed that the arginine kinase reaction proceeds near equilibrium in vivo, then the observed phosphoarginine, arginine, and MgATP concentrations may be used to calculate the sarcoplasmic concentration of free MgADP. From ^{31}P NMR studies on purified lobster arginine kinase, Rao et al. (1976) found K_{eq} for arginine kinase to be 0.10 at pH 7.25, 12 °C, and pMg 3 [K_{eq} is defined as the ratio ([MgADP][phosphoarginine])/([MgATP][L-arginine])]. If it is assumed that the enthalpy for this reaction is similar to that of creatine kinase (Woledge, 1972), i.e., 14 kJ/mol, the corrected K_{eq} at 20 °C would be 0.07. Given the measured intracellular concentrations of phosphoarginine (60 mM), MgATP (6.7 mM), and L-arginine (5.5 mM), then the effective concentration of free sarcoplasmic MgADP will be 0.043 mM. Similar calculations, based on the assumption that the in vivo creatine kinase reaction proceeds at equilibrium, have indicated that free [MgADP] is 0.02 mM in frog skeletal muscle (Gadian et al., 1981) and 0.037 mM in rat skeletal muscle (Veech et al., 1979). Given this calculated value of free [MgADP], the apparent free [inorganic phosphate] $\simeq$ 0.7 mM, and the measured [MgATP] = 6.7 mM in resting barnacle muscle, then the cytosolic free energy of ATP hydrolysis may be calculated to be −61.95 kJ/mol (assuming intracellular pH = 7.3, pMg $\geq$ 3, temperature = 20 °C, and $\Delta G°$ = −31.81 kJ/mol).

In summary, these experiments demonstrate that single muscle cells isolated from barnacle depressor muscle are well

suited for in vivo studies of energy metabolism by ^{31}P NMR. Precise quantitation of intracellular phosphometabolite concentrations is not complicated by heterogeneity of either cell type or metabolic state. The metabolic stability of this preparation should be greatly prolonged by addition of a system for continuous perfusion of the cell with fresh medium. Furthermore, this invertebrate muscle possesses a tonic rather than twitch-type nature and can maintain steady contractions for many minutes in response to sustained depolarization; this feature will facilitate the measurement of changes in phosphometabolites during episodes of high energy turnover. Finally, the capacity to microinject these cells with normally membrane-impermeable phosphonucleotide analogues may add a new dimension to the use of ^{31}P NMR as a probe of phosphate transfer reactions in intact cells.

Acknowledgments

We thank Dr. Mildred Cohn for her continuous encouragement and for many useful discussions in the planning and performance of these experiments. Thanks are also due to Dr. Truman Brown for his suggestions concerning design of the single-cell receiver coil, to James Engle for his help in constructing and tuning the receiver coil, and to Dan Brannen for preparation of the manuscript.

Registry No. ATP, 56-65-5; ADP, 58-64-0; phosphoarginine, 1189-11-3; phosphate, 14265-44-2; L-arginine, 74-79-3.

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