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^{51}V NMR Study of Vanadate Binding to Myosin and Its Subfragment 1†

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ABSTRACT: The binding of various forms of vanadate to myosin and myosin subfragment 1 (S-1) was studied by ^{51}V NMR at increasing vanadate concentrations between 0.06 and 1.0 mM. The distribution of the various forms of vanadate in the solution depended on the total concentration of vanadate. At low concentrations, the predominant vanadate form was monomeric, while at high concentration, it was tetrameric. The presence of myosin or S-1 in the solution produced a significant broadening of the signal of each form of vanadate, indicating that all of them bind to the proteins. Addition of ATP, which does not affect the ^{51}V NMR spectra in the absence of proteins, causes their significant alteration in the presence of myosin or S-1. The changes, which include the broadening of the signal of the monomeric and the narrowing of the signal of the oligomeric vanadate forms, indicate that more monomeric and less oligomeric vanadate binds to the proteins in the presence than in the absence of ATP. Irradiation by near-UV light in the presence of vanadate cleaves S-1 at three specific sites—at 23, 31, and 74 kDa from the N-terminus. The cleavages at 23 and 31 kDa are specifically inhibited by the addition of ATP. The vanadate-associated photocleavage of S-1 also depends on the total concentration of vanadate; it is observed only when the concentration of vanadate is at least 0.2 mM. This was also the lowest concentration at which oligomeric vanadate was detected in the ^{51}V NMR spectra. From the parallel concentration dependence of the photocleavage and the appearance of the tetrameric vanadate, it is concluded that photocleavage occurs only when tetrameric vanadate binds to S-1.

Myosin and actin are two major proteins of muscle whose interaction, coupled with the hydrolysis of ATP, is the molecular basis of contraction. The "head" segment, called subfragment 1 (S-1),[1] of myosin contains two distinct sites (Mueller & Perry, 1962) responsible for actin and nucleotide binding. The driving force of contraction arises as local deformations, forced by ATPase events at the nucleotide binding site, and is conducted through S-1 to the actin binding site, and there compels changes in the myosin–actin relationship (Botts et al., 1984, 1989). The binding of various intermediates of the ATPase cycle induces different structural changes and affects the affinity between actin and myosin. The occupancy of the actin binding site also causes structural changes and influences the lifetime of the intermediates of ATP hydrolysis. These results imply that there is a two-way communication between the nucleotide and the actin binding sites and that S-1 serves as a transducer in the process (Morales & Botts, 1979).

The mechanism of the myosin-catalyzed ATP hydrolysis, because of its functional significance, has been studied in great detail, and the intermediates of the hydrolysis have been described [for a review, see Taylor (1979)]. The predominant steady-state intermediate of the ATP cycle is the M·ADP·P_i complex (M denotes myosin) whose half-life is several seconds. The complex can be further stabilized by substituting phosphate (P_i) with vanadate (V_i), which increases the half-life of the complex to a couple of days (Goodno, 1979). This stable complex, which contains ADP and V_i in a "trapped" form (Okamoto et al., 1986), has proved useful in localizing the nucleotide binding site on S-1. Vanadate, which is considered to be a good analogue of phosphate (Lindquist et al., 1973), was used together with UV irradiation studies to detect the phosphate binding site(s) on S-1. Recently, it has been shown that the S-1 polypeptide chain is cleaved at 23 and 74 kDa from the N-terminus (Mocz, 1989; Cremo et al., 1988). The 23-kDa cleavage site is at Ser-180 (Cremo et al., 1989), and this serine residue participates in the "consensus" ATP binding site of myosin (Walker et al., 1982). The fragment containing

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[1] Abbreviations: S-1, subfragment 1 of myosin; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; LB, line broadening parameter for the exponential multiplication of free induction decay (FID).

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the 1–180 stretch has been isolated and renatured, and its actin binding has been characterized (Muhlrad, 1990).

^{51}V NMR studies have proved to be highly informative in systems of biological relevance. Due to the exceptionally high NMR sensitivity of the ^{51}V isotope [high natural abundance (99.76%), large magnetic moment, and rapid quadrupolar relaxation in solution], resolvable spectra can be obtained in a matter of minutes. ^{51}V NMR studies on the binding of vanadate oligomers to sarcoplasmic reticulum (Csermely et al., 1985), peroxidase (Vilter & Rehder, 1987), apotransferrin (Butler et al., 1987), 6-phosphogluconate dehydrogenases (Crans et al., 1990), and other biologically related molecules reveal a delicate equilibrium among the various forms of vanadate. This equilibrium depends on kinetic parameters and solution conditions (ionic strength, concentration, pH, temperature). The ^{51}V NMR approach was applied to S-1 in preliminary studies (Cremo et al., 1988; Cremo & Wilcott, 1990) and showed that of the various forms of vanadate in solution essentially only the tetrameric form binds to S-1. Because of the importance of vanadate in simulating phosphate binding, we decided to study the V_i binding of myosin and S-1 at various V_i concentrations, in the presence and absence of ATP, and to relate the results of the binding studies to the findings of the V_i-associated photocleavage of S-1. We conclude that (1) mono-, di-, and tetrameric vanadate all bind to myosin and S-1; (2) the presence of ATP reduces the binding of tetrameric and dimeric vanadate while it increases the binding of monomeric vanadate; and finally (3) photocleavage occurs only when tetrameric vanadate is bound to S-1.

Materials and Methods

Reagents. ATP, ADP, sodium orthovanadate, deuterium oxide, chymotrypsin, ammonium sulfate, and HEPES were of the best grade (Sigma). SDS and electrophoresis reagents were purchased from Bio-Rad. All other chemicals were of reagent grade.

Proteins. Myosin was prepared from the back and leg muscles of rabbit, according to Tonomura et al. (1966). S-1 was prepared by digesting myosin filaments with chymotrypsin (Weeds & Taylor, 1975). The S-1 was concentrated, and traces of EDTA were carefully removed by precipitation with 2.5 volumes of cold saturated ammonium sulfate followed by four consecutive dialyses twice against 100 volumes of 100 mM KCl and 10 mM Tris-HCl, pH 8.0, and twice against 100 volumes of 30 mM KCl and 10 mM HEPES, pH 7.0. The last dialyzing solution also contained 10% deuterium oxide, for the NMR lock system. Myosin and S-1 concentrations were estimated by assuming $A(1\%)$ at 280 nm of 5.5 and 7.5, respectively. Molecular masses of myosin and S-1 were taken as 500 and 115 kDa, respectively.

Irradiation of S-1. This was carried out in 2 mM $MgCl_2$, 30 mM KCl, 10 mM HEPES, and 0.06–1.0 mM V_i in the presence or absence of 1 mM ATP under the same ionic conditions as the NMR measurements. The samples were irradiated on ice by a UV transilluminator (U.V.P. Inc) with near-ultraviolet light (peak 365 nm) for 10 min.

SDS–PAGE. Electrophoretic analyses of the samples were performed on 7–18% polyacrylamide gradient gels. The peptide bands were visualized by staining with Coomassie blue. Molecular weights of the peptide bands were estimated by comparing their electrophoretic mobilities with those of authentic markers.

NMR Measurements. NMR measurements of ^{51}V were performed on a Varian VXR 300s spectrometer, at 78.86 MHz, interfaced with a Sun-3 computer, and equipped with a 5-mm multinuclear probe. T_1 measurements of mono-, di-, and tetravanadate revealed values of $<10^{-2}$ s, thus allowing a total accumulation time of 0.05 s per spectrum for a 90° pulse angle, and no relaxation delay. The rapid relaxation times assured the accuracy of signal integration measurements. Typically, 0.8 mL of samples was used, 5000–10000 transients were accumulated (~5–10 min), the spectral width was 8 kHz, and the data memory size was 16K; 10- or 50-Hz exponential line broadening was used before Fourier transformation was performed (a 20-Hz line broadening function was used for all drawn figures). The sample temperature was 18 °C. Assignments of the various vanadate signals were taken from Csermely et al. (1985). The chemical shifts are reported relative to the external reference standard $VOCl_3$ (0 ppm).

Results

Effect of Vanadate Concentrations on the Composition of Various Forms of Vanadate in Solutions. Aqueous vanadate solutions contain complex mixtures of mono- and oligovanadates. The relative amounts of the various forms of vanadate present in a solution depend on the total concentration of vanadate, the pH, the temperature, and the ionic composition of the experimental buffer or medium. We have employed two different buffer systems: for myosin, 0.5 M KCl, 10 mM HEPES, and 2 mM $MgCl_2$, pH 7.0, and for myosin subfragment 1, 30 mM KCl, 10 mM HEPES, and 2 mM $MgCl_2$, pH 7.0 (it is necessary to use a high ionic strength buffer in the case of myosin because it is not soluble at low ionic strength). The relative amounts of the mono-, di-, and tetravanadates (which were the main vanadate species in these mixtures) in the buffers were calculated from NMR measurements (Figure 1; for the NMR spectra of various total vanadate concentrations in buffers, see the left panel of Figures 2 and 3). The amount of the tetrameric form, at a given total vanadate concentration, was much higher in the myosin than in the S-1 buffer, which indicated that high ionic strength, specifically the high KCl concentration, shifted the equilibrium in favor of the tetrameric vanadate. The composition of oligomers in the buffer determined the outcome of any interaction between the vanadate and a macromolecule (Csermely et al., 1985).

^{51}V NMR Study on the Binding of the Various Ionic Forms of Vanadate to Myosin and S-1. Preliminary studies using ^{51}V NMR (Cremo et al., 1988; Cremo & Wilcott, 1990) have concluded that essentially only the tetrameric form of the vanadate binds to S-1, whereas the monomeric and dimeric forms do not bind. Our experiments indicated that although the tetrameric form exhibited the largest apparent change in the line width ($v^{1/2}$, the width of the NMR signal at half-height in hertz, which depends on, among other factors, the extent of association of the observed ligand with a macromolecule and the viscosity of the solution), other forms also exhibited a line broadening upon addition of myosin or S-1 to the vanadate solution (Figures 2 and 3). The line width of the various forms of vanadate in the presence of myosin or S-1 was much broader than would be expected from the change in solution viscosity due to the proteins. The myosin solution was far more viscous than the S-1 solution. Nevertheless, at the same tetravanadate concentrations, the line width of the tetravanadate signal associated with S-1 was ~20% broader than that observed for the tetravanadate signal associated with myosin. In the presence of S-1 (95 μM), the broadening of the monovanadate signal, compared to its width in the buffer alone, ranged from 31 Hz at 200 μM total vanadate concentration (LB = 10 Hz) to 15 Hz at 1000 μM and only 6 Hz at 1500 μM. The apparent K_D for monomeric vanadate, as-

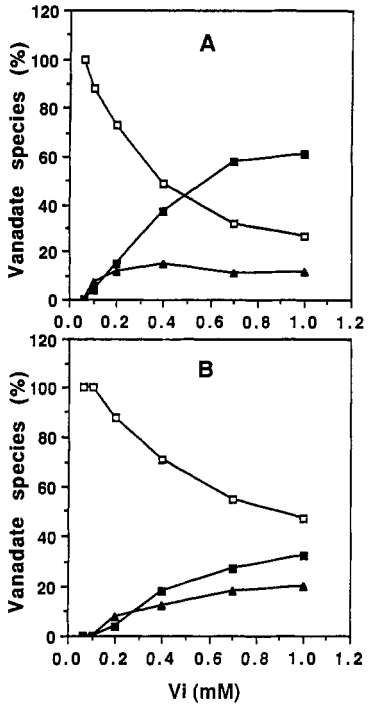

FIGURE 1: Relative concentrations of the different forms of vanadate in myosin and S-1 buffers, at various total vanadate concentrations. The areas of the NMR signals were analyzed and their concentrations expressed as a percent of total vanadate, plotted against the total vanadium concentration: (A) in myosin buffer (0.5 M KCl, 10 mM HEPES, and 2 mM $MgCl_2$, pH 7.0); (B) in S-1 buffer (30 mM KCl, 10 mM HEPES, and 2 mM $MgCl_2$, pH 7.0). (□) Monovanadate; (▲) vanadate dimers; (■) vanadate tetramers.

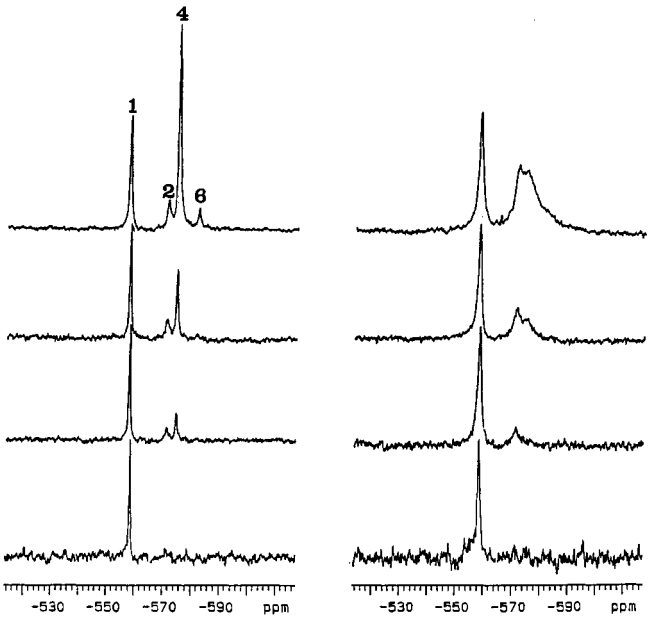

FIGURE 2: ^{51}V NMR spectra of various concentrations of vanadate, in the presence and absence of myosin. (Left panel) Distribution of the different forms of vanadate at various total vanadate concentrations, in myosin buffer. The bands are designated as follows: (1) monovanadate; (2) divanadate; (4) tetravanadate; (6) hexavanadate. (Right panel) As in the left panel but in the presence of 50 μM myosin (100 μM myosin head). Total vanadate concentrations are (from bottom to top) 60, 200, 400, and 1000 μM.

suming a rapid-exchanging complex and a single site in the fast-exchange limit, was calculated to be ~300 μM. It was analyzed by using a reformulation of the Swift and Connick equations by Marshall and Carruthers (1981). Signal broadening was detected also for the di-, tetra-, and hexa-

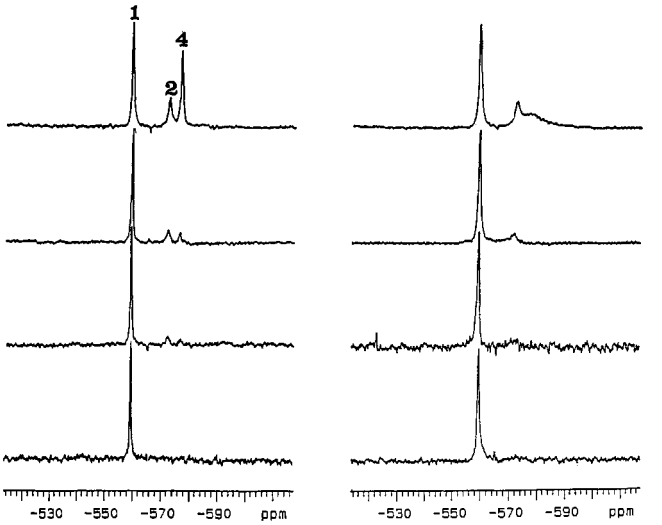

FIGURE 3: ^{51}V NMR spectra of various concentrations of vanadate, in the presence and absence of S-1. (Left panel) Distribution of the different forms of vanadate at various total vanadate concentrations, in S-1 buffer. The bands are designated as follows: (1) monovanadate; (2) divanadate; (4) tetravanadate. (Right panel) As in the left panel but with the presence of 95 μM S-1. Total vanadate concentrations are (from bottom to top) 60, 200, 400, and 1000 μM.

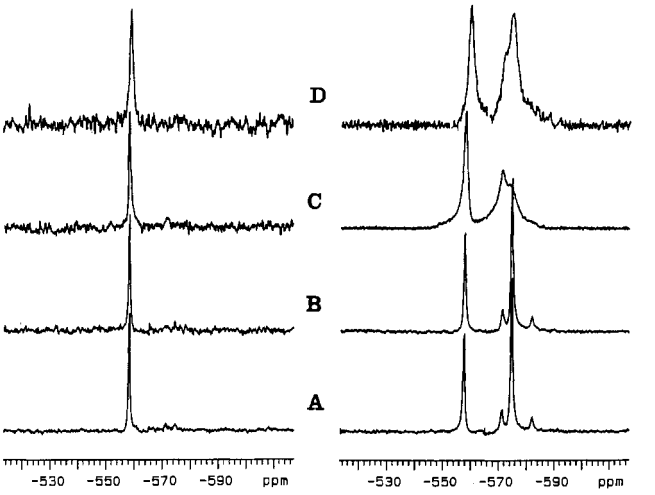

FIGURE 4: Effect of ATP on the binding of the different ionic forms of vanadate to myosin. ^{51}V NMR spectra of samples containing 100 μM (left) and 700 μM (right) vanadate. The solutions also contain (A) myosin buffer alone (See Figure 1), (B) 1 mM ATP, (C) 50 μM myosin, and (D) 50 μM myosin and 1 mM ATP, all in myosin buffer.

vanadates, thus indicating a complex equilibrium among the vanadate species in solution and the vanadate associated with the binding site(s) on the proteins.

Effect of ATP on the Binding of V_i and Oligovanadates to Myosin and S-1. ATP (1 mM final concentration) was added to protein-free samples of myosin and S-1 buffers containing various concentrations of vanadate. The NMR spectra (Figures 4A,B and 5A,B for myosin and S-1 buffer, respectively) showed no differences in the line widths and chemical shifts between solutions that contain or exclude ATP. Therefore, it was inferred that ATP does not associate with any of the forms of vanadate present in the experimental systems. On the other hand, when ATP (1 mM final concentration) was added to identical experimental systems containing myosin or S-1 and the NMR spectrum was recorded immediately thereafter (Figures 4C,D and 5C,D for myosin and S-1, respectively), every vanadate species was affected. The chemical shift of monovanadate was shifted upfield, 1.0–1.5 ppm for the myosin system and 0.6–0.8 ppm

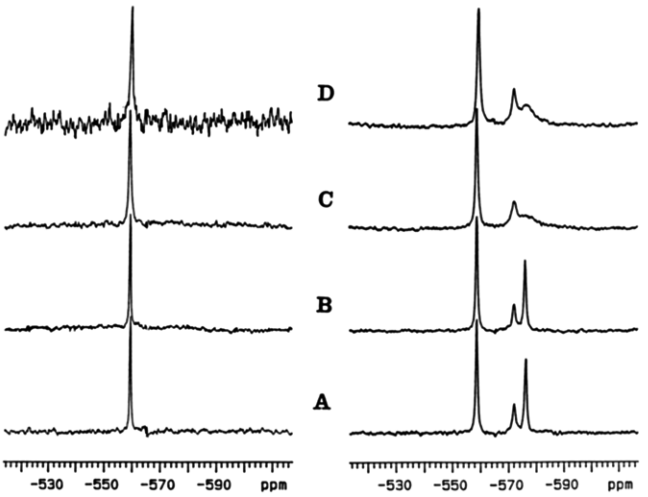

FIGURE 5: Effect of ATP on the binding of the different ionic forms of vanadate to S-1. ^{51}V NMR spectra of samples containing 100 μM (left) and 1000 μM (right) vanadate (A–D). The solutions also contain (A) S-1 buffer alone (see Figure 1), (B) 1 mM ATP, (C) 95 μM S-1, and (D) 95 μM S-1 and 1 mM ATP, all in S-1 buffer.

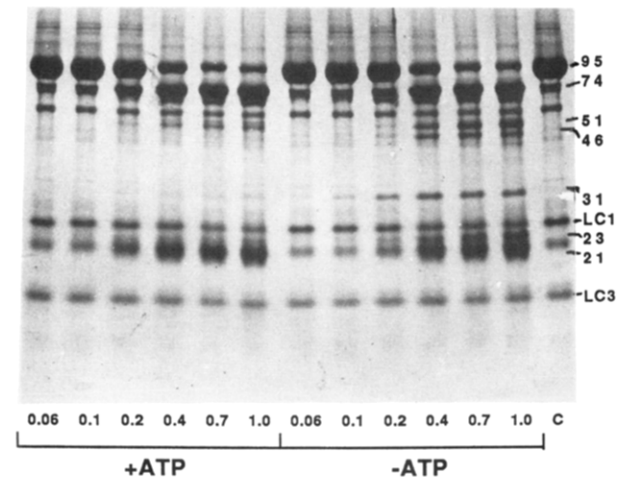

FIGURE 6: SDS gel electrophoresis pattern of S-1 following V_i-dependent photocleavage. 95 μM S-1 was irradiated by near-UV light on ice for 10 min in the presence or absence of ATP as described under Materials and Methods. Horizontal numbers: V_i concentration in millimolar; (C) nonirradiated control S-1. Vertical numbers: apparent molecular masses in kilodaltons, based on electrophoretic mobility.

for the S-1 system, and the line width became 50–60 and 10–35 Hz (LB = 10 Hz) wider for the myosin and S-1 systems, respectively, as compared to the same experimental solutions without ATP. Tetravanadate exhibited a much more profound, but opposite in direction, response on addition of ATP. In both systems, the line width of the tetravanadate signal narrowed about 150 Hz (LB = 50 Hz) from its width in the absence of ATP (~650–750 Hz). The signal of the divanadate also narrowed (because of the overlap between the di- and tetravanadate signals, it was impossible to obtain an accurate line width for the divanadate signal). At high vanadate concentrations, a broad signal (>1000 Hz) could be observed in the region of hexameric vanadate in the presence of protein. The line width of this signal was also affected by ATP addition. These results implied that in the presence of ATP, the affinity of the monovanadate form for the proteins increases while that of the tetravanadate diminishes. This may explain the inhibitory effect of ATP on the photocleavage of myosin and S-1 by UV irradiation in the presence of vanadate. ADP which exerts a much reduced inhibiting effect on the photocleavage compare to ATP (data not shown) has less effect on the NMR spectrum. Upon the addition of ADP to the protein–vanadate solution, no change was observed in monovanadate line width, and the narrowing of the di- and tetravanadate signals was less profound than with the addition of ATP (data not shown).

Vanadate-Associated Photocleavage of S-1. In order to correlate the results of the NMR binding studies with those of the V_i-dependent photocleavage, S-1 was irradiated by UV light in the presence of V_i under the conditions used for the ^{51}V NMR measurements. Following irradiation, which was performed in the presence or absence of ATP, the products of the photocleavage were analyzed by SDS-PAGE (Figure 6). The electrophoretic patterns indicated that the 95-kDa heavy chain of S-1 was cleaved only when the concentration of V_i was 0.2 mM or higher; the LC1 and the LC3 light chains were hardly affected by the irradiation. The extent of cleavage in the presence of 0.2 mM V_i was small, and two new bands, 31 and 23 kDa (according to electrophoretic mobility), appeared when the irradiation was carried out in the absence of ATP. With the increase in $[V_i]$, the extent of cleavage increased, and additional products, 51, 46, and 21 kDa, appeared in the gel pattern, together with a strong 74-kDa band, which

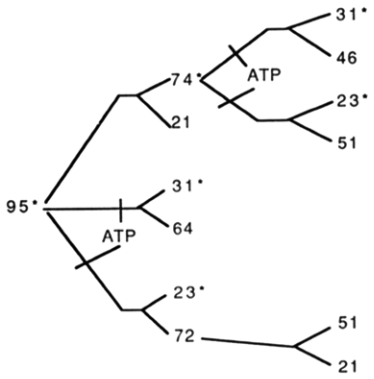

FIGURE 7: Scheme of the V_i-dependent photocleavage of the S-1 heavy chain. ATP, steps inhibited by ATP; asterisks, fragments containing the N-terminus of the S-1 heavy chain; numbers, mulecular masses of S-1 heavy chain fragments in kilodaltons.

is an intermediate in the cleavage (Mocz, 1989). On the basis of our results and those of Mocz (1989), a cleavage scheme is presented in Figure 7. The presence of ATP during the irradiation significantly altered the cleavage process; the 23-, 31-, and 46-kDa bands practically were absent, and the first cleavage products observed at low concentration of V_i were the 21-kDa fragment and the 74-kDa intermediate and not the 31- and 23-kDa fragments, which were found in the absence of ATP. It seems that ATP specifically inhibited the cleavage of the S-1 heavy chain at 23 and 31 kDa from the N-terminus, leaving only the site at 74 kDa susceptible to V_i-dependent photocleavage (Figure 6).

Discussion

The vanadate binding of myosin and S-1 was studied by ^{51}V NMR in the concentration range of 0.06–1.0 mM V_i, at pH 7.0. It is known that at pH 7.0, the various forms (monomer and oligomers) of vanadate are in equilibrium and that the fraction of the oligomeric, especially the tetrameric, vanadate sharply increases with an increase in total $[V_i]$ (Csermely et al., 1985). In our studies, practically no oligomeric vanadate was observed when the V_i concentration was less than 0.2 mM in the low ionic strength (S-1) buffer or less than 0.1 mM in the high ionic strength (myosin) buffer. The increase in total vanadate concentration led to the emergence of dimeric and tetrameric vanadate species; at 1 mM V_i, tetrameric vanadate

became the predominant form, especially in the high ionic strength (myosin) buffer (Figure 1). In the presence of myosin or S-1, the signals of all three vanadate species broadened significantly. The change in the line width indicates that mono-, di-, and tetravanadates alike associate with myosin or S-1. The dependence of the signal width on the concentration of V_i indicates a real ligand–protein association and a fast equilibrium between the free and bound states, at least in the case of the monomeric vanadate, where the signal width can be measured with reasonable accuracy. Cremo et al. (1988) and Cremo and Wilcott (1990) in their preliminary reports stated that only the tetrameric species of vanadium binds to S-1 and that no binding of either the monomeric or the dimeric species was detectable. Although we agree with their finding that the binding of the tetrameric form is essential to the UV photocleavage process, our finding suggests a more complex dynamic equilibrium among the various forms of vanadate. This equilibrium involves both the distribution among the various vanadate species in solution and their association with the proteins.

Not only the distribution of the various forms of vanadate but also the extent of the vanadate-associated photocleavage of S-1 depends on the total V_i concentration. No cleavage was observed when the total concentration of V_i was less than 0.2 mM, and even at this concentration, the extent of cleavage was very limited; however, it started to increase sharply at higher $[V_i]$. Basically, a sigmoidal concentration dependence was obtained similar to that observed by Mocz (1989). It is significant that below 0.2 mM V_i, only the monomeric vanadate species is present and it is at this concentration that the oligomeric vanadates were first observed. With further increase in $[V_i]$, the fraction of the tetrameric vanadate increases sigmoidally, as does the extent of photocleavage. It should be noted that although the extent of photocleavage is proportional to the concentration of the tetravanadate, it depends also on the ionic strength of the buffer. At higher ionic strengths, the efficiency of the tetravanadate as a mediator for photocleavage diminishes. Our findings show a lower response of myosin (which is in a higher ionic strength buffer than S-1) to V_i-dependent photocleavage, as compared to that of S-1 (unpublished results). This finding may coincide with the fact that the association of tetravanadate to S-1 is stronger than to myosin, as can be concluded from the broader signals for the tetravanadate in the presence of S-1, as compared to that in the presence of myosin. From the parallel increase in the fraction of the tetrameric vanadate and the extent of photocleavage of S-1, we conclude that photocleavage occurs only when tetrameric vanadate binds to specific sites on S-1. Similar conclusions were reached by Cremo et al. (1989) and Cremo and Wilcott (1990).

Since vanadate is considered to act as a phosphate analogue (Lindquist et al., 1973), we may learn about possible phosphate binding sites by describing the sites of the vanadate-associated photocleavage. The site which is extensively cleaved at high (>0.2 mM) concentration of vanadate (Figure 6) is located 74 kDa from the N-terminus of S-1. This region contains a cluster of positively charged residues which could easily bind the tetrameric vanadate polyanion. The finding of Chaussepied et al. (1986) that an isolated 30-kDa C-terminal fragment of S-1, including this region, can bind polyphosphate supports the foregoing assumption. This region also participates in an actin binding site of S-1 (Mornet et al., 1979; Sutoh, 1983), but it does not have a direct role in ATP hydrolysis (Chaussepied & Morales, 1988). According to our unpublished observations, actin specifically inhibits the vanadate-associated photocleavage at this site. All these results point to the possibility that the polyphosphate binding site, located 74 kDa from the N-terminus, participates in the regulation of the myosin–actin interaction. The two other sites, which are cleaved in the presence of vanadate, are located 23 and 31 kDa from the N-terminus of S-1. The exact position of the 23-kDa cleavage site was assigned to Ser-180 by Cremo et al. (1988). This residue is located on the 178–185 sequence which forms the "consensus" ATP binding site of myosin and is homologous to the polyphosphate binding site of a number of nucleotide binding enzymes (Walker et al., 1982). The 31-kDa cleavage site (Dan-Goor et al., 1990) is located on a highly conserved region of the S-1 heavy chain, a fact that suggests its possible functional significance. Both cleavages at 23 and at 31 kDa are specifically inhibited by ATP. In the presence of 0.2 mM V_i and in the absence of ATP, the cleavage occurs at the 31- and 23-kDa sites, while in the presence of ATP only the 74-kDa cleavage appears. These observations can be explained by assuming a competition between the 31- and 23-kDa sites, on the one hand, and the 74-kDa site, on the other hand, for the low concentration of tetrameric vanadate present. The 23- and 31-kDa sites have a higher affinity for the tetrameric vanadate than the 74-kDa site. Therefore, the former sites are cleaved when the concentration of the tetrameric form is too low to be effective at all the binding sites. In the presence of ATP, when the binding of tetrameric vanadate to the 23- and 31-kDa sites is inhibited, the tetrameric vanadate is available for binding to the 74-kDa site, and, therefore, the cleavage occurs there.

The addition of ATP, in the presence of myosin or S-1, has a profound effect on the ^{51}V NMR spectrum although it does not affect the spectrum in the absence of the proteins. The lack of change of NMR signals with buffers alone may indicate that ATP, unlike pyrophosphate or phosphate (Gresser et al., 1986), does not react with vanadate at pH 7.0. Therefore, the changes in the NMR spectrum upon addition of ATP, in the presence of protein, are caused by ATP-induced alterations in the myosin or S-1 structure and are not a result of an interaction of ATP with vanadate. It is well-known that the binding and hydrolysis of ATP cause localized structural changes in S-1 which are manifested, among others, in an altered UV absorbance spectrum (Morita, 1967), an increase in tryptophan fluorescence emission (Werber et al., 1972), a change in proteolytic susceptibility (Muhlrad & Hozumi, 1982), and a reduced heat sensitivity (Setton & Muhlrad, 1984). The essence of the ATP-induced change in the NMR spectrum is that the signals of the di- and tetrameric vanadate become narrower, while that of the monomeric vanadate becomes broader, upon addition of ATP. These observations can be interpreted by assuming that the oligomeric species are displaced from the protein, either by direct competition between ATP and vanadate for the binding sites on S-1 or myosin or by localized structural changes caused by the binding of ATP or by both. The broadening of the monomeric vanadate signal is probably due to increased binding of this species to the proteins. This is also caused by ATP-induced structural changes, which may also expose hidden sites capable of binding monomeric vanadate.

In summary, we conclude that all three vanadate species present in solution at pH 7.0 bind to myosin or S-1. However, only the binding of tetrameric vanadate makes the photocleavage possible. This agrees also with the findings in other systems; e.g., Crans et al. (1990) showed that the tetrameric form has a major role in vanadate inhibition of organic phosphate converting enzymes. ATP, which inhibits photo-

cleavage at specific sites, causes structural changes in the myosin head which reduce the binding of the tetrameric species and augment the binding of monomeric vanadate. Finally, the study of vanadate binding by NMR spectroscopy, together with vanadate-associated photocleavage, can contribute to the description of the location and function of the phosphate binding sites on myosin.

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Registry No. ATP, 56-65-5; phosphate, 14265-44-2; vanadate, 37353-31-4.

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