Journal of Biomolecular NMR, 3 (1993) 325–334
ESCOM

J-Bio NMR 123

[2-^{3}H]ATP synthesis and ^{3}H NMR spectroscopy of enzyme–nucleotide complexes: ADP and ADP.V_i bound to myosin subfragment 1

Stefan Highsmith[a,*], Mark Kubinec[b], Devendra K. Jaiswal[c], Hiromi Morimoto[c], Philip G. Williams[c] and David E. Wemmer[b]

[a]*Department of Biochemistry, University of the Pacific, San Francisco, CA 94115, U.S.A.*
[b]*Structural Biology Division and* [c]*National Tritium Labeling Facility, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720, U.S.A.*

Received 6 November 1992
Accepted 5 March 1993

Keywords: Tritium NMR; ATP; Myosin; ATP site; Vanadate

SUMMARY

The synthesis of [2-^{3}H]ATP with specific activity high enough to use for ^{3}H NMR spectroscopy at micromolar concentrations was accomplished by tritiodehalogenation of 2-Br-ATP. ATP with greater than 80% substitution at the 2-position and negligible tritium levels at other positions had a single ^{3}H NMR peak at 8.20 ppm in 1D spectra obtained at 533 MHz. This result enables the application of tritium NMR spectroscopy to ATP utilizing enzymes.

The proteolytic fragment of skeletal muscle myosin, called S1, consists of a heavy chain (95 kDa) and one alkali light chain (16 or 21 kDa) complex that retains myosin ATPase activity. In the presence of Mg^{2+}, S1 converts [2-^{3}H]ATP to [2-^{3}H]ADP and the complex S1.Mg[2-^{3}H]ADP has ADP bound in the active site. At 0 °C, 1D ^{3}H NMR spectra of S1.Mg[2-^{3}H]ADP have two broadened peaks shifted 0.55 and 0.90 ppm upfield from the peak due to free [2-^{3}H]ADP. Spectra with good signal-to-noise for 0.10 mM S1.Mg[2-^{3}H]ADP were obtained in 180 min. The magnitude of the chemical shift caused by binding is consistent with the presence of an aromatic side chain being in the active site. Spectra were the same for S1 with either of the alkali light chains present, suggesting that the alkali light chains do not interact differently with the active site. The two broad peaks appear to be due to the two conformations of S1 that have been observed previously by other techniques. Raising the temperature to 20 °C causes small changes in the chemical shifts, narrows the peak widths from 150 to 80 Hz, and increases the relative area under the more upfield peak. Addition of orthovanadate (V_i) to produce S1.Mg[2-^{3}H]ADP.V_i shifts both peaks slightly more upfield without changing their widths or relative areas.

INTRODUCTION

The power of ^{1}H NMR spectroscopy to determine protein–ligand structures in solution is compromised when proteins larger than about 40 kDa are investigated, because the number of

*To whom correspondence should be addressed.

non-exchangeable hydrogens becomes too large, and individual signals cannot be resolved and assigned. To avoid this problem, we have synthesized the chemically native probe molecule [2-^{3}H]ATP in order to use its ^{3}H NMR signal to obtain information about the structures of the ATP binding sites. ^{3}H has several advantages for NMR spectroscopy (Al-Rawi et al., 1974; Newmark et al., 1990). It is structurally equivalent to ^{1}H and behaves identically for cases that do not involve the substituted position in a chemical reaction. Tritium is a spin-1/2 nucleus with an exceptionally high magnetogyric ratio (1.07 times that of hydrogen). These properties enhance its detectability, especially when broad peaks are anticipated. The low natural abundance of ^{3}H reduces background signals to a negligible level. The chemical shifts for ^{3}H are nearly identical to those of ^{1}H, which allows one to use the vast literature of proton NMR results to interpret the tritium data. Tritium is a weak β-emitter and can be handled safely by using routine procedures for radioisotopes.

In muscle and several other motile systems, the protein–actomyosin complex converts the chemical energy available from the hydrolysis of ATP into mechanical energy, or work. The mechanical elements of this energy transducing system are the proteins actin and a portion of myosin named subfragment-1 (S1[1]), which consists of a heavy chain (~95 kDa) and two light chains (~20 kDa each). S1 can be prepared from myosin by limited proteolysis, which degrades the so-called DTNB light chain, and purified as a complex of the heavy chain and one or the other of the so-called alkali light chains (A1 or A2). Preparations of S1A1 or S1A2 retain full ATPase activity and bind to actin. The local structural changes of the ATP binding site on S1, as MgATP is hydrolyzed and its products dissociate, are transduced into global structural changes of the acto–S1 complex. The global structural changes have macroscopic mechanical consequences.

The details of the local structural changes of the nucleotide-ATP binding site and their connections to the macromolecular structural changes are largely unknown. Nonetheless, several aspects of the process are known or are widely accepted. The isolated S1 portion of myosin is sufficient for transducing ATP chemical energy into motion in the presence of actin (Toyoshima et al., 1987). The S1 light chains do not appear to be required for interaction with ATP or actin (Wagner and Giniger, 1981). The ATP and actin binding sites on S1 are about 5 nm apart (Tokunaga et al., 1987; Kasprzak et al., 1989). Both sites can be occupied simultaneously (Highsmith, 1976), and are connected by linkage through the S1 protein matrix (Botts et al., 1989). Changes in the occupancy of the ATP binding site cause changes in the structure of the distant actin binding site (Yamamoto, 1990; Highsmith and Murphy, 1992). It is likely that force is generated by the acto–S1–nucleotide complex during the transition of the ATP hydrolysis products from MgADP.P to MgADP bound at the ATP binding site on S1 (Taylor, 1979). Finally, chemical reactions involving S1 and nucleotide analogs have implicated Trp^{130} (Okamoto and Yount, 1985; Sutoh, 1987; Pate et al., 1991), Ser^{180} (Cremo et al., 1989) and Ser^{324} (Mahmood et al., 1989) as residues that are part of, or near to, the ATP binding site on S1. The residues Trp^{130} and Ser^{180} are also part of or near to the portion of S1 primary sequence that has been identified as a conserved

---

Abbreviations: S1, myosin subfragment-1; A1, myosin alkali light chain-1; A2, myosin alkali light chain-2; S1A1, S1 heavy chain-alkali light chain 1 complex; S1A2, S1 heavy chain-alkali light chain 2 complex; DTNB, 4,4'-dithiobis(2-nitrobenzoic acid); ATP, adenosine 5'-triphosphate; OAc, acetate; V_i, orthovanadate; P_i, orthophosphate; D, deuterium; Mops, 3-(*N*-morpholino)propane sulfonic acid; EDTA, ethylenediamine tetraacetic acid; NMR, nuclear magnetic resonance; δ, chemical shift; ppm, parts per million.

structure that exists in several ATP binding proteins and enzymes, and is involved in binding the γ-phosphoryl of ATP (Walker et al., 1982).

To date in the literature, only the chemical reactions above have provided information about the molecular structure of the ATP binding site, but this approach has potential problems. The reactive species are analogs of ATP and may not bind as ATP itself does. Another potential risk is that the chemical reactions may select for the most reactive neighboring residue rather than the nearest residue to the ligand. As an example, the Trp^{130} that reacts with the photolabile ATP analog (Okamoto and Yount, 1985) is also the most chemically reactive tryptophan of S1 (Peyser et al., 1990). In the present study, the ^3H NMR signal from [2-^{3}H]ADP bound to S1 is used to investigate the active site structure and the effects of S1 light chain composition, temperature changes and orthovanadate binding on active site–nucleotide interactions.

MATERIALS AND METHODS

2-Br-ATP synthesis

2-Br-ATP was selected as the halogenated precursor for catalytic tritiodehalogenation to [2-^{3}H]ATP. The synthesis started with guanosine 2',3',5'-triacetate, which was reacted with phosphoryl chloride to form the 6-chloro derivative in 62% yield (Robins and Uznanski, 1986); the addition of phosphoryl chloride to the reaction mixture at −20 °C instead of at room temperature improved the yield to 84%. Subsequent deaminative bromination with *t*-butyl nitrite and bromoform resulted in 2-bromo-6-chloro-9-β-(2',3',5'-tri-*O*-acetyl D-ribofuranosyl)purine (Nair and Richardson, 1982), which on ammonolysis gave 2-bromo-adenosine (Montgomery and Hewson, 1964). The barium salt of 2-bromo-adenosine 5'-monophosphate was obtained by the reaction of phosphoryl chloride in triethyl phosphate, as described for the general synthesis of nucleotide 5'-monophosphates (Eckstein and Goumet, 1978). The monophosphate was converted to the tributylammonium salt and reacted with anhydrous tributylammonium pyrophosphate in the presence of 1,1-carbonyldiimidazole to form 2-bromo-ATP tributylammonium salt from which 2-bromo-ATP tetrasodium salt was obtained by treatment with sodium perchlorate in acetone (Hoard and Ott, 1965). [2-^{3}H]ATP was synthesized from 2-Br-ATP as described in the Results section.

Proteins and chemicals

Complexes of S1 with either alkali light chain-1 or -2 bound to the heavy chain were prepared according to the method of Weeds and Taylor (1975) with some modification. Data are shown here for myosin prepared from dorsal muscle taken from one rabbit and then proteolyzed at 20 °C for 10 min with a 1000:1 mol/mol ratio of myosin to α-chymotrypsin. Other data (not shown) were obtained from S1 that was prepared from mixed dorsal and leg muscle myosins, or from the action of α-chymotrypsin on myofibrils, or from S1 that was purified by size exclusion chromatography (Sephacryl™ S-400). The MgATPase activity at 23 °C was in the 0.05 to 0.07 s^{-1} range in all cases, but as described in the Results section there was some variation in the relative areas of ^{3}H peaks of S1-bound [2-^{3}H]ADP for the various preparations. In all cases, peaks occurred only at the chemical shifts given in Table 1. Only the relative areas changed.

Tritium gas was purchased from EG&G Mound Laboratories, OH. Guanosine and other chemicals used in the synthesis of 2-Br-ATP were from Aldrich Chemical Co., and were used

without further purification. Phosphoryl chloride was distilled freshly before use. Chemicals used to prepare buffers were commercial reagent grade and were used without purification.

NMR measurements

A General Electric model GN500 NMR spectrometer was modified in house to enable adequate amplication of the irradiating pulses at 533 MHz and equipped with a ^3H probe that held 10-mm tubes. The free induction decay was collected after a 30-μs (50°) pulse, with a 2-s delay between pulses. ^1H-coupled ^3H NMR spectra typically required 6000–8000 scans (3–4 h) to achieve satisfactory signal-to-noise ratios for 1.8-ml samples of 100–200 μM [2-^3H]ADP (23 Ci ^3H/mmol ADP) bound to S1. A frequency lock was maintained using 10% D_2O in aqueous solutions. The free induction decay signals were multiplied by an exponential apodization function using 10-Hz line broadening before Fourier transformation.

Fresh samples of S1A1 or S1A2 were concentrated in buffer containing 200 mM KOAc, 10 mM Mops, 5 mM $MgCl_2$, 0.05 mM EDTA and 10% D_2O at pH 7.0 by centrifugation against a 30 000 molecular weight cut-off centricon (BioRad™) filter. [2-^3H]ATP was added in a small volume (3–10 μl) before beginning an NMR measurement to provide [[2-^3H]ADP] to saturate the ATP binding site. Samples were prepared in a restricted area and transported to the spectrometer in plugged teflon NMR tube liners that were made air-tight using parafilm.

RESULTS

Synthesis of [2-^3H]ATP

2-Br-adenosine 5'-triphosphate was tritiodehalogenated by using carrier-free tritium gas in pH 8.6 phosphate buffer in the presence of PdO (Evans et al., 1974). The product [2-^3H]ATP was purified by HPLC using a Supelco LC-18 column, eluting with 0.10 M diammonium phosphate (pH 6.0). Proton and phosphorous NMR spectra (not shown) confirmed that the product was ATP. The C-2 proton signal at 8.20 ppm was greatly reduced. Tritium NMR at 0 °C (Fig. 1A)

TABLE 1
[2-^3H]ATP, S1.Mg[[2-^3H]ADP AND S1.Mg[2-^3H]ADP.V_i CHEMICAL SHIFTS[a] AND LINE WIDTHS[b]

Temperature	Sample	δ (ppm)	Line width (Hz)
0 °C	[2-^3H]ATP	8 20	2
	S1.Mg[2-^3H]ADP	7.65	150
		7.30	150
	S1.Mg[2-^3H]ADP.V	7.40	150
		7.13	150
20 °C	[2-^3H]ATP	8.17	2
	S1.Mg[2-^3H]ADP	7.55	80
		7.29	80
	S1.Mg[2-^3H]ADP V	7.43	80
		7.22	80

[a] Chemical shifts in ppm obtained at 533 MHz and 0 or 20 °C, referenced from $^3H^1HO$ at 5.06 and 4.85 ppm, respectively.
[b] Line widths have about ±20% uncertainty. Conditions are described in Figs. 1 and 2.

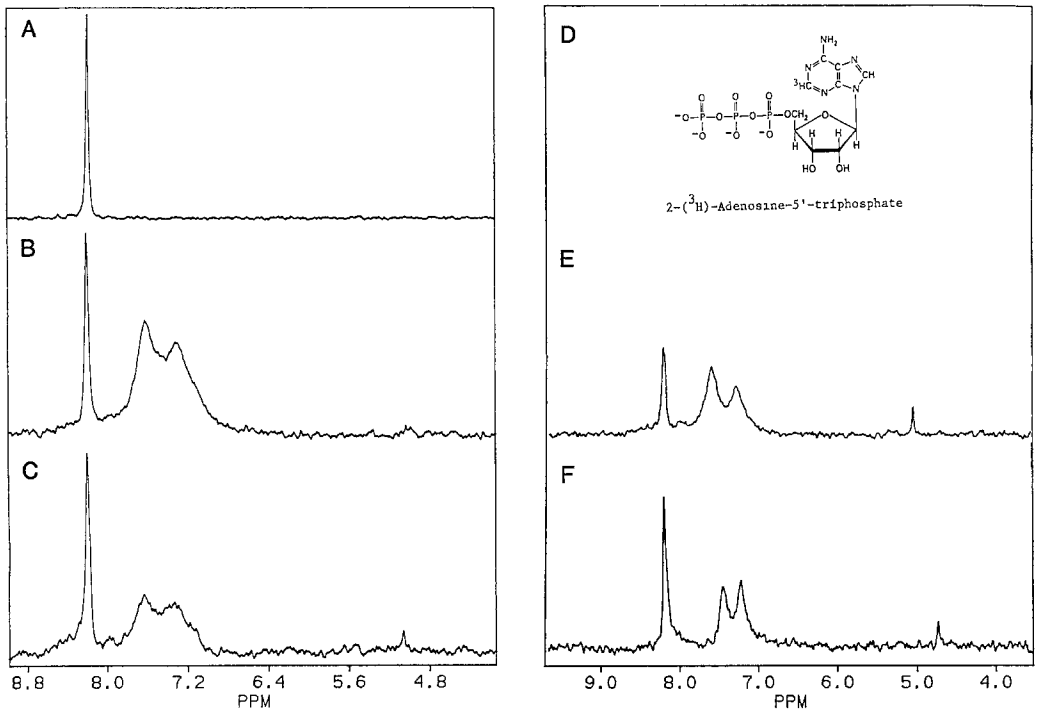

Fig. 1. Tritium NMR spectra. Solutions are 200 mM KOAc, 5 mM $MgCl_2$, 0.05 mM EDTA, 10 mM Mops (pH 7.0) with (A) 0.18 mM [2-^{3}H]ATP; (B) 0.22 mM [2-^{3}H]ADP and 0.20 mM S1A1; (C) 0.20 mM[2-^{3}H]ADP and 0.18 mM S1A2 at 0 °C, or (E) 0.19 mM [2-^{3}H]ADP and 0.17 mM S1A1/S1A2 at 0 °C; or (F) at 20 °C. The small variable peak near 5 ppm in some spectra is due to $^3H^1HO$. The [[2-^{3}H]ADP] is based on the assumption that all the ATP added was converted to ADP by S1, and has a larger than usual experimental uncertainty because very small volumes of stock [2-^{3}H]ATP solutions were diluted into much larger sample volumes. Spectrometer and solutions are more fully described in the Materials and Methods section.

showed a single peak at 8.20 ppm and in some preparations a much smaller peak at 8.52 ppm, which results from substitution of tritium at the C-8 position. The purified [2-^{3}H]ATP typically had a specific activity of 23 Ci/mmol at the 2-position, indicating that the substitution approaches stoichiometric (28.72 Ci/mmol). Spectra were easily obtained using 10 μM [2-^{3}H]ATP solutions. Its structure is shown in Fig. 1D. [2-^{3}H]ATP was stored as 15–20 mM solutions in D_2O at −70 °C. The C-2 position was stable at least for weeks, but the ^{3}H in the C-8 position was lost by exchange with water within several days, as expected (Elvidge et al., 1973).

Reaction of [2-^{3}H]ATP with S1

S1 was mixed with [2-^{3}H]ATP at 0 °C in 200 mM KOAc, 5 mM $MgCl_2$, 0.05 mM EDTA, 10 mM Mops (pH 7.0). Under these conditions ATP is hydrolyzed completely to ADP, and the free and bound ligand is ADP (Bagshaw and Trentham, 1974). The substitution of tritium for hydrogen at the 2-position will not change the kinetics of the hydrolysis, and S1.Mg[2-^{3}H]ADP is the only nucleotide–S1 complex present at the time of NMR signal acquisition. ATP and ADP 2-position tritons have the same chemical shift. The reduced narrow peak at 8.20 ppm (Fig. 1) is

due to free [2-^{3}H]ADP. The two broad peaks upfield in the spectrum at 7.65 and 7.30 ppm (Fig. 1 and Table 1) are due to S1.Mg[2-^{3}H]ADP. The positions, widths and relative intensities of the two peaks were the same regardless of which alkali light chain was selected during S1 purification (Figs. 1B and C). This absence of alkali light chain effect was observed also when fractions from the anion exchange chromatography preparations of S1A1 and S1A2 were taken from narrow central or leading portions of the well-resolved peaks. The relative amplitudes (but not the chemical shifts) were, however, sensitive to the conditions used to prepare S1. Variations in peak amplitudes from those shown in Fig. 1 occurred when size exclusion rather than ion exchange chromatography was used to purify S1, or when myofibrils were used instead of myosin to prepare S1. In all cases, the ratios of the areas under the peaks at 7.65 and 7.30 ppm were in the range of 3:2 to 2:3 at 0 °C. These variations in peak amplitude were not investigated systematically because they do not affect any of the conclusions drawn from the results.

Spectra for S1A1 in the presence of increasing [[2-^{3}H]ATP] were recorded and the ratios of the peak heights at 7.65 and 7.30 ppm are shown in Table 2. The ratio is unchanged, within experimental uncertainty, as the bound [ADP] increases from 25 to 100% saturation, indicating that the two broad peaks represent binding at sites on S1 which have comparable affinities for ADP.

When the temperature was raised from 0 to 20 °C, the free [2-^{3}H]ADP peak was affected only slightly, while the peaks from the bound [2-^{3}H]ADP were shifted farther upfield to 7.55 and 7.29 ppm and narrowed from about 150 Hz to about 80 Hz full width at half height (Table 1). At 20 °C, the area of higher field peak increased, while that of the lower field peak decreased, compared to the areas at 0 °C (Figs. 1E and F). The temperature-induced shift and redistribution favoring the higher field peak are reversible and occur for all original distributions of peak areas at 0 °C.

The S1.Mg[2-^{3}H]ADP.V_i complex

Orthovanadate, Mg^{2+}, ADP and S1 are known to form a very stable complex which is thought to be a transition state analog for the hydrolysis of ATP or an analog for the steady state intermediate MgADP.P_i that is bound to S1 during ATP hydrolysis (Goodno, 1979). When 300 μM orthovanadate was added to solutions of S1.Mg[2-^{3}H]ADP, the signal from the free ligand was unchanged (not shown). The signals from the bound [2-^{3}H]ADP were shifted upfield to 7.40 and 7.13 ppm at 0 °C and to 7.43 and 7.22 ppm at 20 °C (Table 1). That both peaks are shifted by vanadate is strong evidence that both are due to S1.MgADP. Vanadate caused no detectable change in line width or in the relative areas of the two peaks at either temperature (Fig. 2). For

TABLE 2
TITRATION OF S1 ACTIVE SITE

[[2-^{3}H]ATP] / [S1A1]	Ratio of peak heights[a]
0.25	1.2
0.50	1.0
0.75	0.9
1.00	1.2

[a] The ratio of the peak heights at 7.65 and 7.30 ppm for 0.20 mM S1A1 at 0 °C with increasing amounts of [2-^{3}H]ATP added to obtain S1.Mg[2-^{3}H]ADP. Conditions are as in Fig. 1.

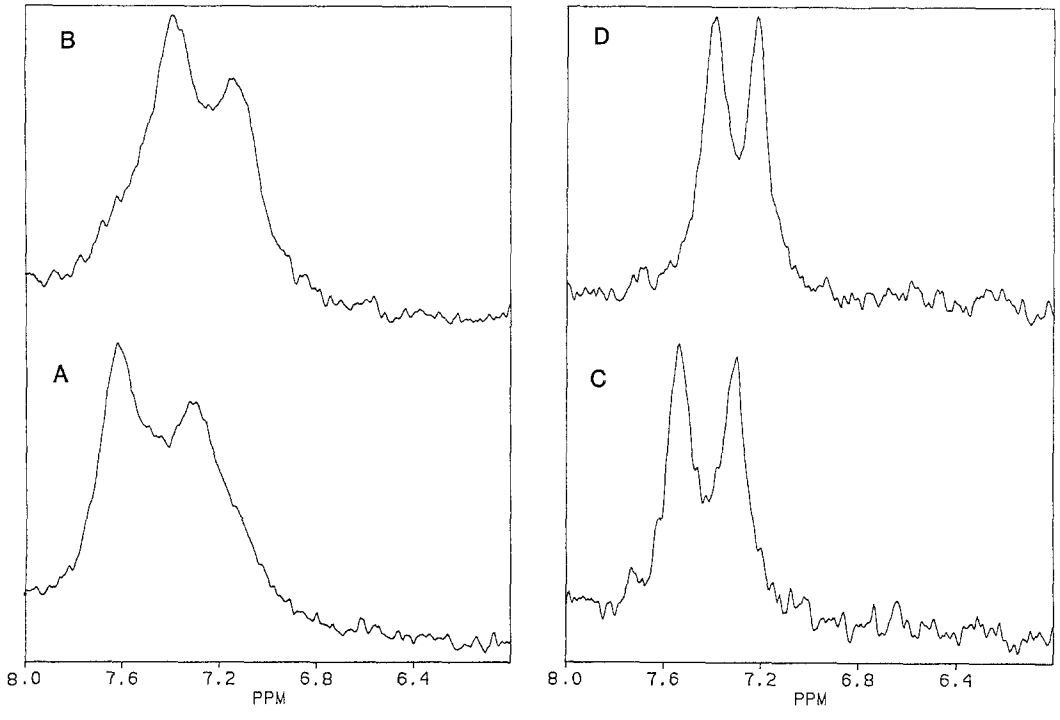

Fig. 2. S1.Mg[2-^{3}H]ADP.V_i. At 0 °C (A) is the ^{3}H spectrum for S1.Mg[2-^{3}H]ADP and (B) is the spectrum for the sample with orthovanadate added to equal the total [ADP]. The results obtained at 20 °C are in (C) no vanadate, and (D) vanadate. Solutions are as described in Fig. 1.

these conditions at 25 °C, the MgATPase activity of S1 is reduced to less than 8% of control within 15 min (data not shown). At either temperature, spectra obtained rapidly after 1 h and after 3 h incubation were not detectably different.

DISCUSSION

The PdO catalysis of the tritiodehalogenation of 2-Br-ATP by $^3H_2$ provides [2-^{3}H]ADP with high specific activity in the 2-position, which after HPLC purification is suitable for NMR studies of ATP–enzyme complexes. The smaller amount (up to 10%) of tritiation at the 8-position which occurred in some syntheses is not a problem for the present work, and if necessary the 8-position can be converted completely back to proton by aqueous exchange with negligible effect at the 2-position. The main product, [2-^{3}H]ATP, is stable for times that are long compared to those needed to make an NMR measurement. After weeks at −70 °C with intermittent thawing and refreezing, spurious ^{3}H NMR peaks were observed at 0.01 to 0.04 ppm upfield from the peak at 8.20 ppm. The source of these peaks was not identified, but may be the products of radiolytic degradation. The spurious peaks were unchanged by the addition of S1, suggesting that they are due to structures that cannot bind to S1, or are present in too low a concentration to bind. All the results reported here were obtained with fresh [2-^{3}H]ATP. The simple 1D ^{3}H NMR spectra

obtained make it possible to assign peaks that cannot be resolved in ^{1}H NMR spectra of S1 complexes (Highsmith et al., 1979; Prince et al., 1981).

Although there are many factors that might contribute to a change in chemical shift upon binding (Jardetzky and Roberts, 1981), the magnitude of the upfield chemical shift of the ^{3}H-signal when ADP binds S1 (Table 1) suggests that an aromatic residue of S1 may be near to the 2-position of the adenine of ADP (Dwek, 1973). This interpretation is consistent with the results of several earlier studies. For instance, circular dichroism results obtained with heavy meromyosin during steady state hydrolysis of ATP have been interpreted in terms of an aromatic group being near the purine of ATP (Murphy, 1974). In particular, tryptophans have been implicated to be near the bound nucleotide. Trp^{130} has been suggested to be near ATP because it can be chemically modified by ATP analogs (Okamoto and Yount, 1985), although its reactivity may be nonspecific (Peyser et al., 1990). The 3D crystal structure of nucleotide-free chicken skeletal myosin S1 determined by X-ray diffraction appears to have two Trps in the ATP site (Rayment, personal communication). One structural arrangement that is consistent with the above results and the changes in tryptophan absorbance (Morita, 1967) and fluorescence (Werber et al., 1972) that occur when ATP binds to myosin is to have a tryptophan side chain exposed as part of the empty ATP binding site and then covered by the adenine portion of ATP when it binds to the site. This mechanism is consistent also with the observed (Fig. 1) upfield shift for the 2-^{3}H peak of ADP when it is bound to S1.

The existence of two bound species of S1A1 (Fig. 1B) is consistent with two S1 isomers, two S1 conformations or specific and nonspecific ADP binding. The similar apparent affinities for binding at the two apparent sites (Table 2) make nonspecific interactions unlikely. The conditions are such that ADP would be expected to be bound only at the active site. Although S1A1 appears to be homogeneous when analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis (Weeds and Taylor, 1976 and analysis of our S1 preparations, data not shown), isomers cannot be rigorously excluded. However, the reversible change in relative peak intensity that occurs when the temperature is changed between 0 and 20 °C (Figs. 1E and F) is consistent with two conformations of S1 that are in equilibrium, as reported originally by Shriver and Sykes (1981) who observed analogous changes in ^{31}P chemical shifts and peak intensities from the β-phosphoryl group of S1-bound ADP as the temperature was changed. This temperature-dependent transformation between two S1-nucleotide conformations is well documented (Lin and Cheung, 1991 and references therein). The decrease in line width induced by increasing the temperature from 0 to 20 °C is rationalized by the reduced solvent viscosity which reduces the NMR correlation time. Small changes in S1 hydrodynamic behavior would not be detected by the methods used here.

It is remarkable that the relative areas of the ^{3}H peaks at either temperature are also sensitive to the S1 preparation used, as mentioned above. It is clear that the alkali light chains do not affect the spectra (Fig. 1). This result suggests that they do not interact differently with the ATP site, at least in the absence of actin. Perhaps, differences in the heavy chain or in bound residual fragments of the DTNB-light chain that is destroyed during the proteolysis of myosin cause the variable peak areas. In invertebrate muscle, the DTNB-light chain is a regulatory subunit (Kendrick-Jones et al., 1970). The regulatory function is limited in skeletal muscle, but the speculative residual fragments could modulate the equilibrium between the two conformations of S1 and its ATP binding site.

Vanadate binding to form the S1.Mg[2-^{3}H]ADP.V_i complex shifts the peaks further upfield (Fig. 2), but does not appear to change the equilibrium between the two temperature-sensitive S1 conformations. The observation that both peaks are shifted when orthovanadate binds, supports the conclusion that both peaks are due to active site binding. The S1.MgADP.V_i complex is known to be very stable (Goodno, 1979). In experiments using the fluorescent analog of ADP, 1-N^6-ethenoadenine diphosphate, bound to S1, it appeared that vanadate binding enhanced the binding of the nucleotide to S1 (Rosenfield and Taylor, 1984). The vanadate-induced upfield chemical shift (Fig. 2) is consistent with a change in the structural interaction between the adenine portion of ADP and the active site that is caused by vanadate binding. If it is assumed that the observed chemical shift changes upon the binding of ADP to S1 are due to the proximity of a Trp side chain in the active site as speculated above, then the increased chemical shift that occurs when vanadate binds suggests that the spatial relationship between the Trp on S1 and the 2-position of ADP may change for the transition from S1.MgADP.V_i to S1.MgADP. The removal of orthovanadate greatly accelerates the rate of nucleotide dissociation (Goodno, 1979). Based on the ^{3}H NMR data presented here and earlier results on S1 Trp–nucleotide interactions (Morita, 1967; Werber et al., 1972; Bagshaw and Trenthan, 1974; Papp et al., 1992) we propose that a S1 Trp is involved also in binding the vanadate or phosphate of S1.MgADP.V_i or S1.MgADP.P_i. Depending on whether the conformation of the bound nucleotide is syn or anti, one Trp could be near both the 2-position and the V_i or P_i, or two Trps may be involved.

ACKNOWLEDGEMENTS

This work was supported by NIH grants AR37499 and P41 RR01237, DOE grant DE-FG05-86ER75281 and Contract DE-AC03-76SF0098, and NSF grant DMB 86-09035.

REFERENCES

Al-Rawi, J.M.A., Bloxsidge, J.P., O'Brien, C., Caddy, D.E., Elridge, J.A., Jones, J.R. and Evans, E.A. (1974) *J. Chem. Soc. Perkin Trans. 2*, 1635–1639.

Bagshaw, C.R. and Trentham, D.R. (1974) *Biochem. J*, **141**, 331–349.

Botts, J., Thomason, J.F. and Morales, M.F. (1989) *Proc. Natl. Acad. Sci. USA*, **86**, 2204–2208.

Cremo, C.R., Grammer, J.C. and Yount, R.G. (1989) *J Biol. Chem*, **264**, 6608–6611.

Dwek, R.A. (1973) *Nuclear Magnetic Resonance in Biochemistry*, Clarendon, Oxford, pp. 48–54

Eckstein, F. and Goumet, M. (1978) In *Nucleic Acid Chemistry, Vol 2* (Eds., Townsend, L.B. and Tipson, R.S.) Wiley, New York, pp. 861–864.

Elvidge, J.A., Jones, J.R., O'Brien, C., Evans, E.A. and Sheppard, H C. (1973) *J. Chem. Soc., Perkin Trans. 2*, 2138–2141.

Evans, E.A., Sheppard, H.C., Turner, J.C. and Warrell, D.C. (1974) *J. Label Compounds Radiopharm*, **10**, 569–587.

Goodno, C.C. (1979) *Proc. Natl. Acad Sci. USA*, **76**, 2620–2624.

Highsmith, S. (1976) *J. Biol Chem.*, **251**, 6170–6172.

Highsmith, S. and Murphy, A.J. (1992) *Biochemistry*, **31**, 385–389.

Highsmith, S., Akasaka, K., Konrad, M., Goody, R., Holmes, K., Wade-Jardetzky, N. and Jardetzky, O. (1979) *Biochemistry*, **18**, 4238–4244.

Hoard, D.E. and Ott, D.G. (1965) *J. Am. Chem. Soc.*, **87**, 1785–1788.

Jardetzky, O. and Roberts, G.C.K. (1981) *NMR in Molecular Biology*, Academic Press, New York, pp. 379–412.

Kasprzak, A.A., Chaussepied, P. and Morales, M.F. (1989) *Biochemistry*, **28**, 9230–9238.

Kendrick-Jones, J., Lehman, W. and Szent-Gyoryi, A.G. (1970) *Methods Enzymol.*, **11**, 199–203.

Lin, S.H. and Cheung, H.C. (1991) *Biochemistry*, **30**, 4317–4322.
Mahmood, R., Elzinga, M. and Yount, R.G. (1989) *Biochemistry*, **28**, 3989–3995.
Montgomery, J.A. and Hewson, K. (1964) *J. Heterocyclic Chem.*, **1**, 213–214.
Morita, F. (1967) *J. Biol. Chem.*, **242**, 4501–4506.
Murphy, A.J. (1974) *Arch Biochem. Biophys.*, **163**, 290–296.
Nair, V. and Richardson, S.G. (1982) *Synthesis*, 670–672.
Newmark, R.D., Un, S., Williams, P.G., Carson, P.L., Morimoto, H. and Klein, M.P. (1990) *Proc. Natl. Acad. Sci USA*, **87**, 583–587.
Okamoto, Y. and Yount, R.G. (1985) *Proc. Natl Acad. Sci. USA*, **82**, 155–159.
Papp, S., Eden, D. and Highsmith, S. (1992) *Biochim Biophys Acta*, **1159**, 267–273.
Pate, E., Nakamaye, K.L., Franks-Skiba, K., Yount, R.G. and Cooke, R. (1991) *Biophys. J.*, **59**, 598–605.
Peyser, Y.M., Muhlrad, A and Werber, M.M. (1990) *FEBS Lett.*, **259**, 346–348.
Prince, H.P., Trayer, H R., Henry, G.D., Trayer, I.P., Dalgarno, D.C., Levine, B.A., Cary, P.D. and Turner, C. (1981) *Eur. J. Biochem.*, **121**, 213–219.
Robins, M.J. and Uznanski, B. (1986) In *Nucleic Acid Chemistry, Vol. 3* (Eds., Townsend, L.B. and Tipson, R.S.) Wiley, New York, pp. 144–148.
Rosenfield, S.S. and Taylor, E.W (1984) *J. Biol Chem*, **259**, 11920–11929.
Shriver, J.W. and Sykes, B.D. (1981) *Biochemistry*, **20**, 2004–2012.
Sutoh, K. (1987) *Biochemistry*, **26**, 7648–7654.
Taylor, E.D. (1979) *CRC Crit Rev. Biochem.*, **6**, 103–106.
Tokunaga, M., Sutoh, K., Toyoshima, C. and Wakabayashi, T. (1987) *Nature (London)*, **329**, 635–638.
Toyoshima, Y.Y., Kron, S.J., McNally, E.M., Niebling, K.R., Toyoshima, C. and Spudich, J.A. (1987) *Nature (London)*, **328**, 536–539.
Wagner, P.D. and Giniger, E. (1981) *Nature (London)*, **292**, 560–562.
Walker, J.E., Seraste, M., Runswick, M.J. and Gay, N.J. (1982) *EMBO J.*, **1**, 945–951.
Weeds, A.G. and Taylor, R.S. (1975) *Nature (London)*, **257**, 54–56.
Werber, M.M., Szent-Gyorgyi, A.G. and Fasman, G.D. (1972) *Biochemistry*, **11**, 2872–2883.
Yamamoto, K. (1990) *Biochemistry*, **29**, 844–848.