Myosin Subfragment 1 Hydrophobicity Changes Associated with Different Nucleotide-Induced Conformations†

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ABSTRACT: Myosin subfragment 1 hydrophobicity was found to be sensitive to the occupancy and nature of bound nucleotide at its active site, as shown by changes in elution behavior of unmodified and chemically modified S1 during phenyl hydrophobic chromatography. The elution properties of S1 were unaltered by alkylation of SH1 (Cys-707) with *N*-ethylmaleimide or by covalent bridging between SH1 and SH2 (Cys-697) with *p*-phenylenedimaleimide with trapping of MgADP. Although addition of MgADP or MgATP to the elution buffers had minimal effect on the elution properties of these modified S1 species, the presence of these nucleotides was found to produce differential effects with unmodified S1. With MgADP, where S1 is in the S1*MgADP state, the elution times were decreased slightly, whereas with MgATP, where S1 is primarily in the S1**MgADP·P_i state, the elution times were significantly lowered, indicating reduced accessibility for the immobilized phenyl ligand. Stable S1 ternary complexes, formed with MgADP and various P_i analogues, showed elution times similar to that for S1 in the buffers containing MgATP. Thus, two main classes of nucleotide-induced S1 conformations can be defined according to their interaction with immobilized phenyl. These nucleotide-induced changes in S1 hydrophobicity correlate well with reported changes in radius of gyration of S1 associated with different states of the bound nucleotide [Wakabayashi, K., Tokunga, M., Kohno, I., Sugimoto, Y.; Hamanaka, T., Takezawa, Y., Wakabayashi, T., & Amemiya, Y. (1992) *Science 258*, 443−447], suggesting that the observed hydrophobicity interaction may be measuring accessibility of the immobilized phenyl ligand into a hydrophobic crevice, and that this crevice is closed or tightened when S1 is in the S1**MgADP·P_i state.

Muscle contraction, and other forms of actomyosin based motility, involves coupling the free energy released by ATP hydrolysis by the actomyosin complex, resulting in a conformational change in this protein complex responsible for either force-generation or actin translocation. During the ATP hydrolysis cycle by S1[1] or actoS1, S1 undergoes sequential changes in conformation with force generation being coupled to the transition step, S1**MgADP·P_i to S1*MgADP, with release of P_i while S1 is attached to actin [reviewed in Cooke (1986) and Goldman (1987)]. In the absence of actin, this transition step is rate-limiting, while in its presence it is markedly accelerated. Since the conformational basis for force generation lies in the difference in the structures of these two S1 intermediates, much effort has been expended in characterizing them. Indeed, a large body of evidence based on spectral and chemical studies has established that these two states are structurally different, but the details of these differences remain to be characterized.

The recently solved X-ray crystallographic structure of S1 has laid the framework for the eventual characterization of the structures of these two states (Rayment et al., 1993a). A particularly interesting aspect of this structure is the presence of a narrow cleft, whose fringes contain residues modified by photoaffinity labeling (Yount et al., 1992). The presence of a large hydrophobic cleft had been predicted previously on the basis of fluorescence energy transfer data of S1 between covalently and noncovalently attached fluorophores (Haugland, 1975). Based on the crystallographic data, a model for force-generation has been proposed where force is associated with a transition between a closed state for the nucleotide-binding cleft (associated with the S1**MgADP·P_i state) and an open state corresponding to the S1*MgADP state (Rayment et al., 1993b). Recently, two studies have been reported to test this hypothesis. The fluorescence quenching data of Franks-Skiba et al. (1994) suggest that, although the nucleotide binding pocket can exist in open and closed states when the fluorescent nucleotide analogue is bound, the power stroke apparently is not related to the transition between these states. On the other hand, the fluorescence quenching data of Hiratsuka (1994) indicate that during steady-state hydrolysis the noncovalently bound dye PPBA is shielded from quenching, consistent with closure of the nucleotide-binding cleft.

To obtain additional information concerning the hydrophobic properties of S1 during nucleotide-binding and hydrolysis, we have sought to examine whether the different nucleotide-bound states of S1 can be differentiated by high-performance phenyl hydrophobic chromatography. Since the binding of aromatic hydrophobic molecules to S1 appears

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[1] Abbreviations: S1, subfragment 1 of myosin; NEM, *N*-ethylmaleimide; pPDM, *p*-phenylenedimaleimide; SH1-NEM-S1, S1 modified by *N*-ethylmaleimide at the SH1 thiol; SH1-NEM, SH2-NEM-S1, subfragment 1 modified at both SH1 and SH2 by *N*-ethylmaleimide; pPDM-S1·MgADP, S1 containing trapped MgADP through covalent bridging of SH1 and SH2 by *p*-phenylenedimaleimide; S1*MgADP and S1**MgADP·P_i, the complexes of S1 with MgADP and MgADP·P_i respectively with the number of asterisks indicating the relative tryptophyl fluorescence intensities; Vi, orthovanadate; BeFx and ScFx, fluoroberyllium and fluoroscandium anions, respectively; PPBA, 3-[4-(3-phenyl-2-pyrazolin-1-yl)benzene-1-sulfonylamido]phenylboronic acid; *o*-NODP, 2-[(2-nitrophenyl)amino]ethyl diphosphate; HPLC, high-performance liquid chromatography.

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to occur at a hydrophobic crevice (Haugland, 1975), differences in the binding of these different conformational S1 states to immobilized phenyl groups could be construed to measure changes in phenyl accessibility into this hydrophobic crevice. Hydrophobic chromatography has been previously used to separate the light-chain-based isoforms of S1 (Borejdo et al., 1975), but to our knowledge this technique has not been used to try to differentiate between the different nucleotide conformations of S1 and of modified S1 species in the absence and presence of nucleotide. The present study indicates that S1 hydrophobicity is only slightly lowered when MgADP is bound but that it is markedly lowered under steady-state conditions when S1 is predominantly in the S1**MgADP·P_i state. This finding is consistent with closure of a hydrophobic crevice and reduced accessibility for the immobilized phenyl ligand for the preforce intermediate S1**MgADP·P_i and is in accord with the hypothesis that the power stroke for contraction is associated with the transition from a compact state to a more open one during the transition to the S1*MgADP state (Wakabayashi et al., 1992; Rayment et al., 1993b). Modification of the SH1 thiol by *N*-ethylmaleimide has little effect on the S1 hydrophobicity and renders this property to be insensitive to the binding and hydrolysis of MgATP. On the other hand, the ternary complexes, formed between S1 and MgADP in the presence of P_i analogues such as Vi, BeFx, AlF_4^-, and ScFx, show hydrophobicity characteristics similar to those observed for S1**MgADP·P_i.

MATERIALS AND METHODS

Proteins. Myosin was prepared from the back and leg muscles of a rabbit as described by Godfrey and Harrington (1972). S1 was prepared by digesting myosin filaments with chymotrypsin (Weeds & Taylor, 1975). S1 refers to a mixture of S1A1 and S1A2 unless otherwise indicated in the text. Protein concentration was determined by absorbance using *A* (1%) at 280 nm of 7.5 for S1.

Chemicals. ATP, ADP, Tris, NEM, and $BeCl_2$, were purchased from Sigma. $ScCl_3$ was from Johnson Mathey Electronics. Sodium orthovanadate, pPDM, and $AlCl_3$ were purchased from Aldrich.

o-NODP was synthesized using the procedures reported by Wang et al. (1993) and Nakamaye et al. (1985). [^{14}C]-ADP (specific activity 2.4×10^{13} cpm/mol) was purchased from New England Nuclear.

High-Performance Phenyl Hydrophobic Chromatography. This was done using a Waters HPLC system as previously described (Burke et al., 1986). An LKB Ultrapac HPLC Column [TSK Phenyl-5 PW (7.5×75 mm)] was run with a gradient formed from ammonium sulfate (1.8 M) and sodium phosphate (0.1 M), pH 7.0 (buffer A), and sodium phosphate (0.1 M), pH 7.0 (buffer B). The program used was 100% solvent A for 5 min; linear gradient for 5 min to 56% solvent A; linear gradient for 70 min to 100% solvent B. A flow rate of 1.0 mL/min was used, and the eluent was monitored at 280 nm. S1 (0.8 mL at 0.50 mg/mL, adjusted to 1.8 M ammonium sulfate) was typically injected. $MgCl_2$ (1.0 mM) and either ATP or ADP at 50 μM were added to S1 and to the elution buffers to study the S1**MgADP·P_i and S1*MgADP states, respectively. Typically, the elution times were quite reproducible (±0.2 min) for a given S1 preparation. In the case where four or more components were present, small changes in elution times can be attributable to non-base-line separations of the individual isoenzymic components present.

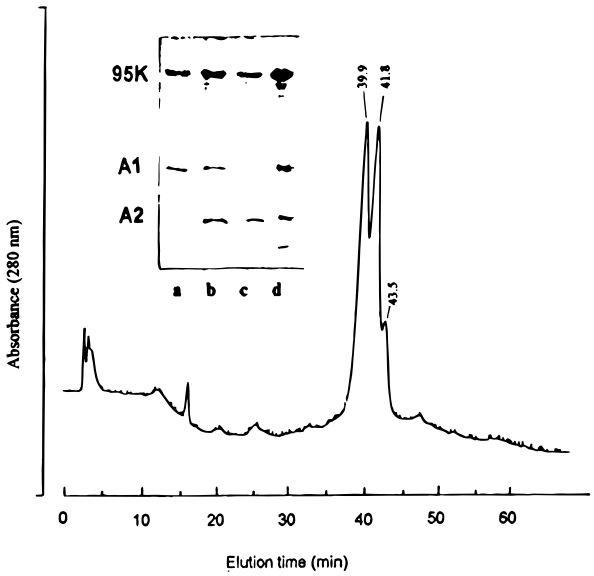

FIGURE 1: High-performance phenyl hydrophobic chromatography of S1. The loading concentration was 0.5 mg/mL. The ordinate is the absorbance at 280 nm, and the abscissa is the elution time. Absorbance is 0.06 units full scale. The flow rate is 1 mL/min, and the gradient used is described in Materials and Methods. (Inset) SDS−PAGE analyses of S1 fractions. Lanes correspond to peaks at (a) 39.9 min, (b) 41.8 min, (c) 43.5 min, and (d) control S1.

Trapping and Modification. The trapping of MgADP, ADP, and *o*-NODP in S1 with phosphate analogues was done as described previously by Werber et al. (1992) and Gopal et al. (1995). The modification of SH1 and/or SH2 by *N*-ethylmaleimide was carried out as reported in the literature (Reisler, 1982). The trapping of MgADP with pPDM by cross-linking of SH1 and SH2 are done as reported by Wells and Yount (1979).

Retention of [^{14}C]ADP. [^{14}C]ADP was trapped in S1 with Vi utilizing the procedure described by Maruta et al. (1993). The excess reagents were removed by gel filtration sedimentation (Penefsky, 1977) using Sephadex G-50 equilibrated in 0.05 M Tris-HCl at pH 7.8. The [^{14}C]ADP trapped S1 was chromatographed as described above, and the entire S1 peaks (32.0 to 39.0 min) were collected for estimation of the trapped [^{14}C]ADP. Aliquots were taken for counting the trapped ADP in S1 and for protein concentration determination.

SDS−PAGE. This was done by the procedure of Laemmli (1970) staining of the protein bands was done with Coomassie Brilliant Blue.

RESULTS

High-Performance Phenyl Hydrophobic Chromatography of Unmodified and Modified S1. The elution pattern observed with unmodified S1 alone is presented in Figure 1 from which it is evident that this preparation has been fractionated into two major peaks and one minor shoulder with elution times of approximately 39.9, 41.8, and 43.6 min, respectively. The SDS−PAGE electrophoretograms of these fractions (Figure 1, inset) show that the first fraction has only the A1 light chain, while the second major peak has A2 as the major and Al as a minor light chain component. The minor shoulder has A2 as the only light chain component

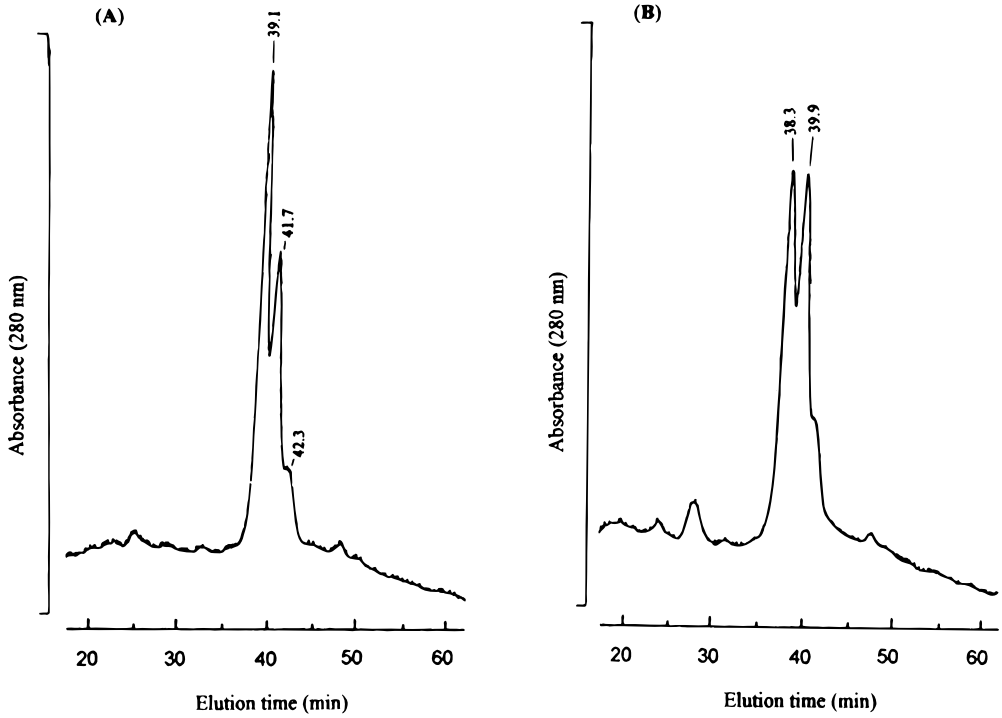

FIGURE 2: High-performance phenyl hydrophobic chromatography of NEM-modified S1. (A) The SH1-NEM S1 and (B) SH1-NEM, SH2-NEM-S1 under the same conditions used for S1. Absorbance full scale is 0.06.

suggesting that the separation appears to be associated with the different light chain isoforms of the S1 preparation. The effect of modification at the SH1 thiol by *N*-ethylmaleimide on the elution behavior of S1 was next investigated, since alkylation of SH1 (and SH2) are known to alter the ATPase function of the protein. The resulting chromatogram is shown in Figure 2A, and the profile is similar to that observed for unmodified S1 with similar elution times for the two major fractions (39.1 and 41.7 min) indicating that this modification causes little if any change in the S1 hydrophobicity properties. Modification at both SH1 and SH2 by *N*-ethylmaleimide brought on a small lowering of the hydrophobicity as indicated by the slightly lower elution times of the two fractions given in (Figure 2B and Table 1). These results suggested that modifications of S1 at either SH1 or at both SH1 and SH2 cause only minor, if any, changes in the hydrophobicity of the protein. It was of interest next to examine to what extent the hydrophobicity of S1 is affected by the nature of the nucleotide bound at the ATPase site.

High-Performance Phenyl Hydrophobic Chromatography of Unmodified and Modified S1 in the Presence of Nucleotides. To examine the effect of bound nucleotide on the hydrophobicity of S1, the elution behavior of S1 was examined in the same buffers supplemented with 1 mM Mg^{2+} together with either 0.05 mM ADP or ATP. It was assumed that these low levels of divalent cations and nucleotides would in themselves have little if any effect on the properties of the immobilized phenyl groups, and any alteration in the elution behavior of the protein would reflect the binding of the particular nucleotide-induced conformational state of the S1. The results of these experiments are shown in the elution patterns of Figure 3A,B. For the elution system supplemented in MgADP, S1 (presumably in the S1*MgADP state) was fractionated into two major peaks eluting at 39.3 and 41.0 min (Figure 3A), which are very similar to the times observed for unliganded S1. On the other hand, for the elution buffer supplemented with MgATP, the S1 (presumably in the S1**MgADP·P_i state) was not well fractionated and eluted as a broad peak at 35.8 min (Figure 3B). These results suggest that for unmodified S1 the hydrophobicity depends on whether the nucleotide binding site is occupied and also on the nature of the nucleotide bound at this site.

Table 1: Elution Times for Unmodified and Modified S1 in the Presence and Absence of Nucleoside and Nonnucleoside Di- and Triphosphates and for S1 Ternary Complexes

protein and conditions	peak 1 (min)	peak 2 min)	peak 3 (min)	peak 4 (min)	peak 5 (min)
S1	39.9	41.8	43.5		
S1 + MgATP (buffer)	35.8				
S1 + MgADP (buffer)	39.3	41.0			
S1 (SH1-NEM)	39.1	41.7	42.3		
S1 (SH1-NEM)MgATP (buffer)	39.1	40.9			
S1 (SH1-NEM)MgADP (buffer)	39.7	41.2			
S1 (SH1-NEM, SH2-NEM)	38.3	39.9			
S1 (SH1-NEM, SH2-NEM) MgATP (buffer)	37.7	39.7			
S1 (SH1-NEM, SH2-NEM) MgADP (buffer)	38.3	39.9			
S1A2	41.6				
S1 A2 MgADP·Vi	35.7				
S1·MgADP·Vi	33.8	36.1	38.4		
S1 + S1MgADP·Vi	33.4	35.7	40.0	42.5	
S1·MgADP·BeFx	34.1	36.2	38.9		
S1·BeFx*	39.9	41.7			
S1·MgADP· ScFx	33.7	36.1	38.6	40.1	41.8
S1·ADP· ScFx	33.5	36.0	38.6	41.5	
S1·MgADP·pPDM	39.9				
S1·MgO-NODP·Vi	33.9	36.0	38.4		

To obtain further insights on these nucleotide-dependent changes in the S1 hydrophobicity as manifested by the lower elution times, similar experiments were also done with SH1-NEM-S1 and SH1-NEM, SH2-NEM-S1. For SH1-NEM-S1, contrary to the situation occurring with unmodified S1, the addition of MgADP or MgATP to the elution buffers had essentially no effect on the elution times of the two major

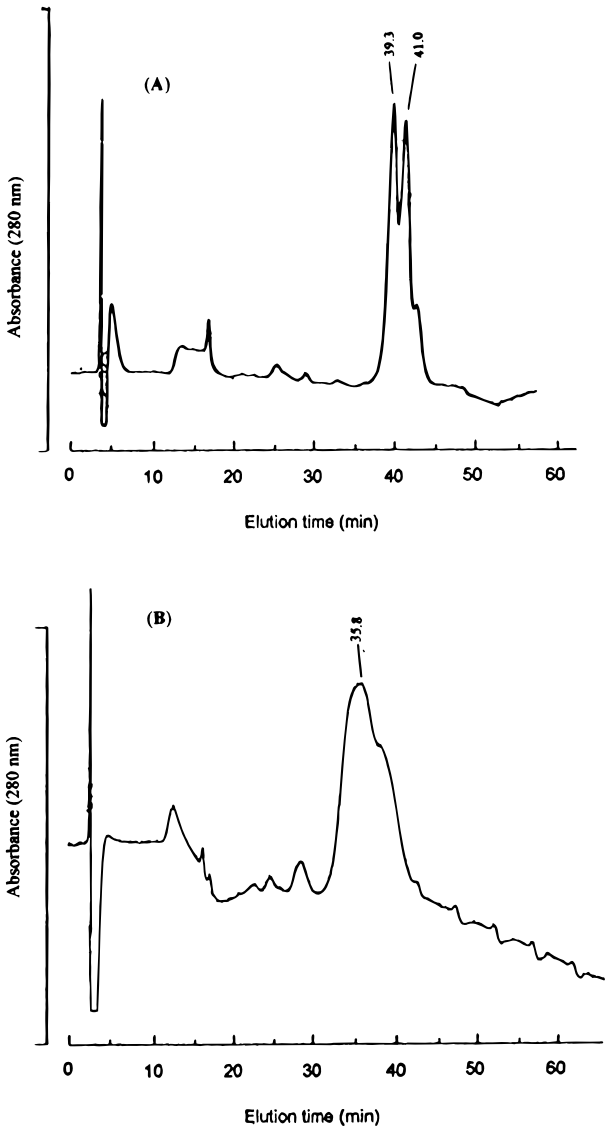

FIGURE 3: High-performance phenyl hydrophobic chromatography of S1 in the presence of MgADP and MgATP. (A) The binding of MgADP to S1 in the presence of 1 mM $MgCl_2$ and 50 μM ADP in the buffers. (B) The binding of MgATP to S1 in the presence of 1 mM $MgCl_2$ and 50 μM ATP in the buffers. Absorbance full scale is 0.06.

fractions, as shown in Table 1. These data imply that the binding of MgADP or MgATP to the SH1-NEM-S1 does not alter the hydrophobicity of the modified S1 and underscore the conclusion that the nucleotide-induced changes in hydrophobicity observed with unmodified S1 reflect marked differences in the S1 structure in the presence of substrate and product. For the doubly modified S1, little if any effects were also observed in the elution times with nucleotides present (Table 1).

High-Performance Phenyl Hydrophobic Chromatography of Ternary MgADP Complexes of S1 Stabilized with Vi or BeFx and of S1 Containing Trapped MgADP through Covalent Bridging of SH1 and SH2 by pPDM. The results shown in the previous two sections indicate that changes in the hydrophobic properties of unmodified S1 accompany the nucleotide-dependent changes in its conformation associated with intermediates of the S1MgATPase cycle. It was of interest, therefore, to examine the hydrophobic characteristics of S1 species containing trapped MgADP to determine if they also showed changes in hydrophobicity compared to unliganded S1 and, if so, whether their hydrophobicities were similar to those observed for either the S1*MgADP or S1**MgADP·P_i states. Modification of S1 with pPDM in the presence of MgADP caused a decrease in the resolution of the S1 which eluted as a broad peak at 39.8 min (Table 1). On the other hand, elution of the ternary complex S1·MgADP·Vi resulted in fractionation of the S1 species into two major and one minor fraction eluting at 33.8, 36.1, and 38.4 min, respectively (Figure 4A). These elution times are markedly shorter than those observed for the corresponding fractions of unmodified and unliganded S1 noted earlier and suggest that the formation of this ternary complex also lowers the hydrophobicity of S1. To further ensure that these changes in elution times were reflective of alterations in the protein's structure and not due to variations in the properties of the column, a mixture of unmodified S1 and this ternary complex were also fractionated, and the results are shown in Figure 4B. In this case four major peaks can be discerned with elution times of about 33.4, 35.7, 40.0, and 42.5 min, indicating that the two early eluting fractions do result from S1 ternary complex formation. The S1 ternary complex formed with MgADP and BeFx was next examined and the elution pattern observed showed two major fractions eluting at 34.1 and 36.2 min (Table 1) similar to the results obtained with the Vi stabilized ternary complex described above. Elution of a mixture of the Vi and BeFx stabilized ternary complexes gave two major fractions eluting with essentially the same times noted above for each separately (data not shown). Thus, the hydrophobicities of the ternary complexes formed with either Vi or BeFx appear to be the same within experimental error.

The elution behavior of the S1 ternary complex formed with ScFx and either MgADP is shown in Figure 5. Two major fractions eluting at 32.9 and 35.3 min followed by three minor fractions eluting at 37.9, 39.3, and 41.0 min can be discerned. The two major fractions most likely correspond to the ternary complexes, while the minor peaks probably reflect unliganded S1 due to decomposition of these complexes, which have been shown to have lower stability than those formed with Vi and BeFx (Gopal & Burke, 1995).

The ternary complex formed using AlF_4^- as the P_i analogue was also examined with another S1 preparation, and this complex was found to elute as two major fractions at 35.8 and 38.0 min with a very minor fraction eluting at about 42.3 min. This latter fraction, in most likelihood, represents some free S1, since, for this preparation, S1 alone eluted as two major fractions at 41.5 and 42.9 min. Therefore, the S1 ternary complex formed with AlF_4^- also has lowered hydrophobicity than that for S1 alone.

It should be noted that variations in elution times between the different forms of ternary complexes most likely reflect incomplete (non-base-line) separation of the individual components resulting in partial overlap of some of them. However, despite these variations, it is evident that S1 species, in which both MgADP and P_i (or P_i analogue) are bound at the active site, have significantly lower elution times corresponding to decreased hydrophobicity.

Since the presence of bound MgADP to unmodified S1 causes little if any change in the elution times of the S1 isoenzymes, the decreased elution times observed for the ternary complexes suggest that the nucleotide is still trapped by the P_i analogue in these fractions. It was of interest, therefore, to examine whether the S1 fractions eluting at

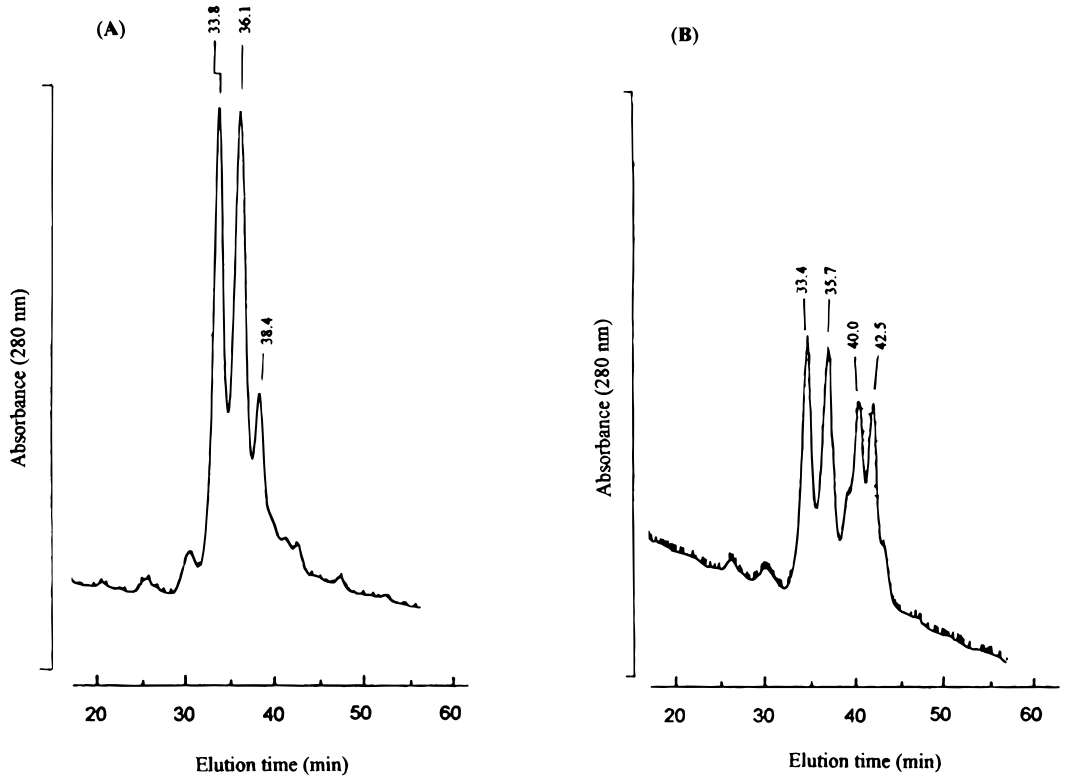

FIGURE 4: High-performance phenyl hydrophobic chromatography of S1·Mg·ADP×b7Vi complex. (A) The ternary complex formed by S1 in the presence of MgADP and Vi. (B) A mixture of S1·MgADP·Vi complex and S1. Absorbance full scale is 0.06.

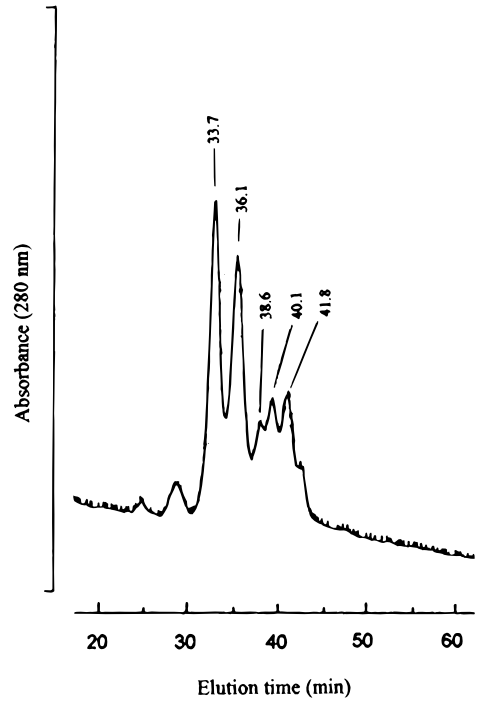

FIGURE 5: High-performance phenyl hydrophobic chromatography of S1·MgADP·ScFx and S1·ADP·ScFx complexes. The ternary complex formed by S1 in the presence of MgADP and ScFx. Absorbance units at the scale indicated is 0.06.

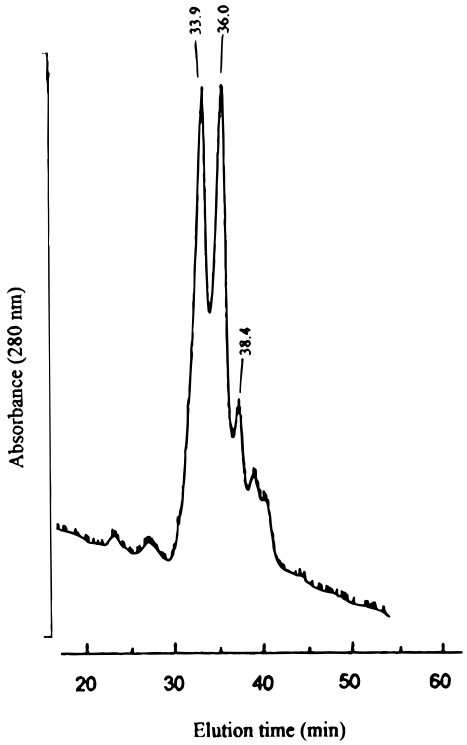

FIGURE 6: High-performance phenyl hydrophobic chromatography of S1·Mg*o*-NODP·Vi complex. The ternary complex formed by S1 in the presence of Mg, *o*-NODP, and Vi with the gradients as in Materials and Methods. Absorbance full scale is 0.06.

earlier times than the unliganded S1 contained trapped nucleotide. This was done by trapping [^{14}C]ADP in S1 with BeFx and then determining whether radioactivity was associated with these earlier eluting fractions. The results obtained indicated that 0.7 mol of [^{14}C]ADP per mol of S1 was present in these fractions, indicating that these fractions did correspond to the ternary complexes.

High-Performance Phenyl Hydrophobic Chromatography of Ternary Mg−Non nucleotide Diphosphate Complexes of S1 Stabilized with Vi or BeFx. Since it is known that some nonnucleotide diphosphate analogues can also be trapped in S1 by Vi or other P_i analogues, it was of interest to determine the hydrophobicity properties of a ternary complex formed

with S1 and the diphosphate corresponding to a nonnucleoside triphosphate known to support tension in skinned fibers (Wang et al., 1993). The elution time(s) for the ternary complex S1·*o*-NODP·Vi complex were found to be the same within experimental error as those for S1·MgADP·BeFx and for S1**MgADP·P_i (Figure 6 and Table 1).

DISCUSSION

The present study was undertaken to determine whether hydrophobic chromatography could be utilized to probe for differences in hydrophobicity of S1 associated with different conformations of the protein induced by nucleotide binding and hydrolysis. The basis of the analysis is that, under high concentrations of ammonium sulfate, proteins tend to bind to immobilized hydrophobic alkyl or aromatic groups through hydrophobic interactions, and that these interactions are weakened at low concentrations of this salt. Therefore, by employing reverse-phase chromatography under a specified elution protocol, the elution behavior of a protein can be characterized. Moreover, it is anticipated that conformational changes in the protein induced either by substrate or product binding or by chemical modification may be manifested in changes in the ability of the protein to interact with the immobilized hydrophobic group, resulting in altered elution times. An increase in elution time would be indicative of a stronger interaction and vice versa.

Earlier work using hydrophobic chromatography showed that fractionations of S1 in terms of the light chain based isoenzymes could be achieved on phenyl-Sepharose CL-4B columns with partial dissociation of the light chains (Borejdo et al., 1984). In this earlier study, the very high halide salt concentrations employed to achieve binding to the latter matrix could conceivably have resulted in partial subunit dissociation of S1. In the present work we have utilized 1.8 M $(NH_4)_2SO_4$ as the major component of buffer A, and it is unlikely that significant subunit dissociation occurs under this condition. For unmodified S1 the reverse-phase phenyl-HPLC results in the fractionation of the S1 into its two light chain isoenzymatic forms (Figure 1). Modification at either SH1 or at both SH1 and SH2 with *N*-ethyl maleimide showed only small decreases in elution times of these two fractions (Figure 2) suggesting that these modifications cause only mimimal perturbation of the S1 hydrophobicity.

The results shown in Figure 3, when MgADP or MgATP were added to the elution buffers, did produce a significant change in the elution times and in the degree of fractionation of unmodified S1 but not with the chemically modified forms of S1. In the presence of MgADP, where S1 is in the S1*MgADP state, it is eluted as two major fractions at slightly shorter times than those observed for the unliganded protein (Figure 3A). Thus, the hydrophobicity of the S1*MgADP intermediate is only slightly lower than that for S1 when the nucleotide binding site is unoccupied. In the presence of MgATP the major species present under steady-state conditions is the S1**MgADP·P_i intermediate, and it is interesting to note that this species is eluted at significantly shorter times but without the resolution observed for unliganded S1 or S1 containing bound MgADP (Figure 3B and Table 1). In the case of SH1-NEM-S1, little change in elution behavior was observed when MgADP or MgATP were added separately to the elution buffers (Table 1). Since it is known that the fraction of S1**MgADP·P_i associated with SH1-modified S1 is very low under steady-state conditions (Sleep et al., 1981), it is not surprising that both MgATP and MgADP show the same (negative) effect on the modified protein. Because of the lack of a significant effect of nucleotide binding on SH1-NEM-S1 elution, it is possible to conclude that the changes, observed with unmodified S1 by the added nucleotides, reflect changes associated with altered S1 conformation and not to artifacts of binding induced by these ligands. Therefore, the technique shows potential of discriminating between the different nucleotide-induced intermediates associated with the S1 MgATPase cycle.

The other point that emerges from the present study is that the binding of the ternary complexes stabilized by Vi, BeFx, ScFx, and AlF_4^- to the immobilized phenyl appears to be similar to that observed with the steady-state intermediate, S1**MgADP·P_i (Table 1). While it is recognized that these ternary complexes are not identical (Maruta et al., 1993; Werber et al., 1992; Phan & Reisler, 1994; Phan et al., 1995), their binding interactions with the immobilized hydrophobic ligand appear to be the same within the sensitivity of the method. Since the same elution characteristics were also observed for the ternary complex of S1 formed with the nonnucleoside diphosphate, o-NODP, and Vi, it may be concluded that the interaction involved is not particularly sensitive to the base moiety of the diphosphate analogue provided it can interact to produce a stable complex with the P_i analogue.

The question then arises as to the structural basis of the interaction between S1 and the immobilized phenyl groups and the basis of its sensitivity to the state of the nucleotide bound. While the exact nature of the binding of S1 to the immobilized phenyl groups remains to be established, it may reflect the ability of the immobilized phenyl group to bind in a hydrophobic crevice of the protein. The immobilized phenyl matrix was chosen since it was felt that the bulkier aromatic group would mimic fluorophores such as ANS which bind stoichiometrically to a hydrophobic pocket in S1. The presence of such a crevice in S1 was proposed some time ago based on fluorescence energy transfer between covalently and noncovalently bound fluorescent probes (Haugland, 1975), and, indeed, the recent crystallographic structure shows a major crevice (Rayment et al., 1993a), containing at its edges, residues involved in nucleotide binding (Yount et al., 1992) together with additional smaller crevices. If it is assumed that S1 binding to the immobilized phenyl groups involves access to a hydrophobic crevice, then the decreased S1 binding, during steady-state MgATP hydrolysis (when S1 is in the S1**MgADP·P_i state), can be attributed to closure of this binding crevice (thereby limiting accessibility for the phenyl group). This explanation would be in accord with the recent observations and conclusions of Hiratsuka (1994) that the hydrophobic binding pocket of S1 associated with the binding of the fluorescent dye PPBA is closed or tightened during substrate hydrolysis, based on decreased acrylamide quenching.

Another interesting feature revealed by the present study is that two main classes of nucleotide bound forms of S1 can be differentiated by this technique. The first class, with higher hydrophobicity, comprises unliganded S1, and S1 containing MgADP alone, either reversibly bound or trapped by pPDM modification. The second class consists of S1 with both MgADP and P_i (or P_i analogues) bound in the

active site (S1**MgADP·P_i) and exhibits weaker hydrophobicity. While the exact nature of this hydrophobic interaction remains to be elucidated, it is evident that the state of the nucleotide in the active site is a major determinant of this interaction. In this respect, it should be pointed out that S1 shape (asymmetry) has also been shown to be sensitive to the state of bound nucleotide (Wakabayashi et al., 1992) and that two major S1 classes could be defined from that work also. In correspondence with the hydrophobicity data, little change in radius of gyration (R_g) of S1 was observed upon binding or trapping MgADP alone with pPDM (from 47.8 to 47.0 and 48.1 Å, respectively) suggesting that entry of MgADP into the active site has only a minimal effect on the overall shape (asymmetry) of the protein, whereas a significant decrease in R_g of S1 was observed when the active site contained both MgADP and P_i (or P_i analogue) (from 47.8 to 45 and 45.9 Å, respectively) indicative of a more compact (less asymmetric) shape for S1. While this correspondence could be entirely fortuitous, it suggests that the higher S1 hydrophobicity associated with S1, S1*MgADP, and pPDM-S1·MgADP may be related to the more asymmetric form of S1 and that the more compact S1 form, induced by the presence of both MgADP and P_i (or P_i analogues) in the active site, favors the weaker S1 hydrophobic state. Since formation of the more compact form of S1 would necessarily lead to closure of cavities in the protein, the decreased hydrophobic binding seen with the compact S1**MgADP·P_i and the ternary complexes formed with MgADP and the P_i analogues would be in accord with decreased accessibility of the immobilized phenyl into a binding crevice.

It is pertinent to note that the recent X-ray crystallographic study by Fisher et al. (1995) indicates that the motor domain of *Dictyostelium discoidium* S1 with MgADP trapped by either BeFx or AlF_4^- shows significant differences in structure. Therefore, the failure to detect significant differences in the hydrophobicity of the corresponding S1 ternary complexes is somewhat surprising. It is possible, however, that the structural differences detected in the smaller motor domains are attenuated in the intact S1 due to the presence of the associated light chain.

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