Differential Scanning Calorimetric Study of the Complexes of Myosin Subfragment 1 with Nucleoside Diphosphates and Vanadate or Beryllium Fluoride†

Andrey A. Bobkov*,‡ and Dmitrii I. Levitsky

A. N. Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow 117071, Russia

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ABSTRACT: It has been recently shown by differential scanning calorimetry (DSC) that the formation of stable complexes of myosin subfragment 1 (S1) with Mg-ADP and orthovanadate (V_i) or beryllium fluoride (BeF_x) causes a global conformational change in the S1 molecule which is reflected in a pronounced increase of S1 thermal stability and in a significant change of S1 domain structure [Shriver, J. W., & Kamath U. (1990) *Biochemistry 29,* 2556−2564; Levitsky, D. I., Shnyrov, V. L., Khvorov, N. V., Bukatina, A. E., Vedenkina, N. S., Permyakov, E. A., Nikolaeva, O. P., & Poglazov, B. F. (1992) *Eur. J. Biochem. 209,* 829−835; Bobkov, A. A., Khvorov, N. V., Golitsina, N. L., & Levitsky, D. I. (1993) *FEBS Lett. 332,* 64−66]. In this work, which continues the previous investigations, we report on a DSC study of the complexes of S1 with various nucleoside diphosphates (NDP). In the absence of V_i or BeF_x the various Mg^{2+}-NDP and Mg^{2+}-PP_i had a similar effect on the S1 conformation. All of them had practically no influence on the temperature of the thermal transition but increased its sharpness. However, in the presence of V_i or BeF_x the effects of Mg^{2+}-NDP complexes were quite different from each other and strongly depended on the base structure of NDP; their effectiveness in inducing conformational changes in S1 and the stability of these complexes decreased in the following order: ADP > CDP ≫ UDP ≫ IDP > GDP. The effects of ADP and CDP were similar while GDP was unable to induce changes in the S1 conformation in the presence of V_i or BeF_x. This order is very similar to that of ability of different NTP to form the predominant intermediate M**-NDP-P_i during myosin Mg^{2+}-NTPase reaction. Thus, DSC studies with the S1-NDP-V_i and S1-NDP-BeF_x complexes offer a new and promising approach to investigate the structural changes which occur in the myosin head due to the formation of M**-NDP-P_i intermediates during Mg^{2+}-NTP hydrolysis.

Muscle contraction and a number of events in cell motility are based on the cyclic interactions of myosin heads with actin which are coupled to myosin-catalyzed ATP hydrolysis. During steady-state ATP hydrolysis a number of discrete intermediates having different spectral properties are formed (Bagshaw & Trentham, 1974; Johnson & Taylor, 1978). The predominant intermediate is the M**-ADP-P_i complex [M** represents the myosin head with enhanced intrinsic fluorescence (Werber et al., 1972)]. This complex has a special significance in the molecular mechanism of muscle contraction, since it is believed to be a key intermediate in the energy transduction process (Johnson & Taylor, 1978). The conformational changes which occur in the myosin head during formation and decomposition of the M**-ADP-P_i complex are, probably, directly responsible for the changes in myosin−actin interaction and, finally, for the power stroke in the mechanical cross-bridge cycle. Thus, there is considerable interest in structural characterization of the myosin head in the M**-ADP-P_i complex.

The use of stable analogues enables the studies on the structure of the myosin head in the M**-ADP-P_i intermediate. A complex of the isolated myosin head, or myosin subfragment 1 (S1),[1] with ADP and orthovanadate ion (V_i) is considered to be a stable analogue of the M**-ADP-P_i complex (Goodno, 1979, 1982; Goodno & Taylor, 1982; Wells & Bagshaw, 1984). A new stable analogue of this intermediate state, the complex of S1 with ADP and beryllium fluoride, has been recently described (Werber et al., 1992; Phan & Reisler, 1992). This complex is more stable than S1-ADP-V_i (Werber et al., 1992; Phan & Reisler, 1992), and it is suggested to have the same actin-binding properties as the M**-ADP-P_i complex (Phan et al., 1993). The structure of the bound beryllium fluoride was investigated by ^{19}F-NMR spectroscopy, and the results indicated that the various complexes (BeF_x) present in free solution, and not only BeF_x as was usually thought, compete for the active site of S1 (Henry et al., 1993).

Recently, we have studied the thermal unfolding of S1 in the S1-ADP-V_i and S1-ADP-BeF_x complexes by means of differential scanning calorimetry (DSC) (Levitsky et al., 1992; Bobkov et al., 1993). We showed that the trapping of ADP by V_i or by BeF_x at the active site of S1 causes a global change of S1 conformation which is reflected in a pronounced increase of S1 thermal stability and in a significant change of S1 domain structure. Also significant increase of thermal stability of S1 and HMM, which was caused by formation of their complexes with ADP and V_i,

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* Author to whom correspondence should be addressed: Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095.

‡ Present address: Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095.

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[1] Abbreviations: S1, myosin subfragment 1; V_i, orthovanadate; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; DSC, differential scanning calorimetry.

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was observed earlier with help of DSC (Shriver & Komath, 1990). Since denaturation of S1 and it complexes with nucleotides was fully irreversible and was accompanied by protein aggregation, the authors could not obtain precise thermodynamic parameters for the unfolding of S1. While, due to special construction of the cells in our calorimeter (Levitsky et al., 1992; Privalov & Potekhin, 1986), we avoided such difficulties and were able to measure thermodynamic parameters for the unfolding of S1 in the complexes with nucleotides and phosphate analogues with relatively small experimental errors.

Thus, the DSC method probes and detects the conformational changes of the whole S1 molecule caused by formation of the S1-ADP-V_i and S1-ADP-BeF_x complexes. These changes, probably, correspond to the structural transitions of the myosin head which are associated with the formation of the M**-ADP-P_i complex during ATPase reaction. In order to lead further support to this hypothesis, we have studied now by DSC the ability of various nucleoside diphosphates (NDP) to induce conformational changes in the S1 molecule in the presence of V_i or BeF_x.

The myosin is known to hydrolyze not only ATP but also a variety of other nucleoside triphosphates (NTP) (Blum, 1955, 1960; Kielley et al., 1956; Werber et al., 1972; Seidel, 1975; Hazzard et al., 1989). However, the ability to form the predominant intermediate M**-NDP-P_i during NTPase reaction is different for various NTP. The enhancment of tryptophan fluorescence in heavy meromyosin accompanying the formation of the M**-NDP-P_i intermediate decreases in the following order: ATP ~ CTP > ITP > UTP > GTP (Werber et al., 1972). Furthermore, a mechanism different from that of S1 Mg^{2+}-ATPase has been proposed for S1 Mg^{2+}-GTPase (Eccleston & Trentham, 1979). In this case the steady-state intermediate consists predominantly of a protein−substrate complex unlike the S1 Mg^{2+}-ATPase reaction which has a protein−products complex as the principal steady-state component.

Therefore, it may be assumed that the conformational changes in S1 molecule caused by the formation of the S1-NDP-V_i and S1-NDP-BeF_x complexes should depend on the ability of the corresponding NTP to form the M**-NDP-P_i complex during myosin Mg^{2+}-NTPase reaction.

MATERIALS AND METHODS

Reagents. ATP, ADP, CDP, IDP, UDP, GDP, sodium orthovanadate, Hepes, NaF, and sodium pyrophosphate were from Sigma Chemical Co. α-Chymotrypsin was from Wornington Biochemicals. Beryllium chloride was obtained from Fluka. All other reagents were of analytical grade. Vanadate stock solution (10 mM) was prepared as described by Goodno (1982).

S1 Preparations. Myosin subfragment 1 (S1) was obtained by digestion of myosin filaments from rabbit skeletal muscles by chymotrypsin (Weeds & Taylor, 1975). S1 concentration was determined spectrophotometrically by using $E^{1\%}_{280nm} = 7.5$ (Wagner & Weeds, 1977).

Preparation of S1-NDP-V_i and S1-NDP-BeF_x Complexes. Trapping of different NDP by V_i or BeF_x was performed by the methods described for the preparation of stable complexes S1-ADP-V_i (Goodno, 1982) and S1-ADP-BeF_x (Werber et al., 1992). The S1-NDP-V_i complexes were obtained by incubation of S1 (1.5 mg/mL) with 1 mM $MgCl_2$, 0.2 mM NDP, and 0.2 mM V_i for 30 min. at 20 °C in a medium containing 30 mM Hepes, pH 7.3. In order to obtain the S1-NDP-BeF_x complexes, S1 (1.5 mg/mL) was incubated for 5 min at 20 °C in 1 mM $MgCl_2$, 30 mM Hepes, pH 7.3, 0.2 mM NDP, and 5 mM NaF; after addition of 0.2 mM $BeCl_2$, the reaction mixture was further incubated at 20 °C for 25 min. The formation of the S1-NDP-V_i and S1-NDP-BeF_x complexes was controlled by measuring the K^+-EDTA-ATPase activity of S1 (Werber et al., 1992). ATPase activity of S1 modified by vanadate or beryllium fluoride in the presence of ADP or CDP did not exceed 10% of the unmodified S1 preparation activity. The activity of S1 modified in the presence of UDP or IDP was about 20−25% of the activity of unmodified S1. On the other hand, the addition of vanadate or beryllium fluoride in the presence of GDP did not change significantly the S1 ATPase activity: in this case K^+-EDTA-ATPase activity of the S1 was inhibited by no more than 15−25%.

Differential Scanning Microcalorimetry (DSC). Calorimetric measurements were carried out on a differential adiabatic scanning microcalorimeter DASM-4 (Biopribor, Puschino, Russia) whose construction and principles have been described elsewhere (Privalov & Potekhin, 1986). Prior to experiments all S1 samples were dialyzed against 30 mM Hepes, pH 7.3, containing 1 mM $MgCl_2$. All measurements were carried out at protein concentration 1.5 mg/mL and a constant heating rate of 1 K/min. Instrumental base lines were determined prior to scanning each sample with two identical measuring cells filled with dialysate. The denaturations were found to be fully irreversible. A base line was subtracted from the data files before analysis. Calorimetric enthalpy, ΔHcal, was calculated from the area under the heat sorption curve. The van't Hoff enthalpy, ΔH_{vH}, was calculated using the equation

$$\Delta H_{vH} = 4R(T_m)^2 C_{p(max)}/\Delta H_{cal}$$

where $C_{p(max)}$ is the maximal C_p of the DSC excess heat capacity curve at the maximal temperature of the transition, T_m. The cooperative ratio, CR, which reflects the cooperativity of the system, was defined as $CR = \Delta H_{vH} / \Delta H_{cal}$ (Bertazzon & Tsong, 1990).

RESULTS

Prior to experiments on the trapping of different NDP by vanadate and beryllium fluoride, we have studied the effects of the NDP binding on the thermal denaturation of S1. Figure 1 shows calorimetric data on the thermally induced unfolding of S1 in the absence (curve a) and presence (curve b) of UDP. It is seen that the binding of UDP to S1 has practically no influence on the temperature of the thermal transition but increases its sharpness. Thus, the binding of UDP to S1 causes a change of S1 conformation which is reflected in a pronounced increase of the cooperativity of the thermal transition (the peak becomes much narrower). In the absence of vanadate or beryllium fluoride the effects of all other NDP were virtually the same (Figures 2 and 3, curves shown by dotted lines) and did not differ from the effect of ADP described earlier (Bobkov et al., 1993). This means that the conformational changes of S1 induced by NDP binding do not depend on the base structure of NDP.

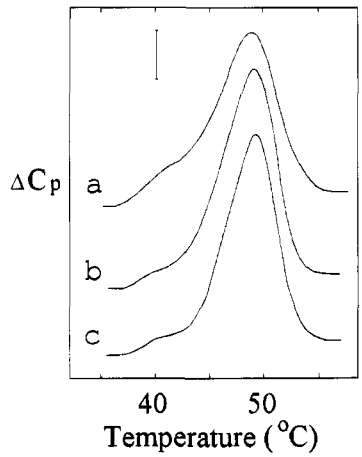

FIGURE 1: Temperature dependencies of excess heat capacity (ΔC_p) for nucleotide-free S1 (curve a), S1 in the presence of 0.2 mM UDP (curve b), and S1 in the presence of 0.3 mM PP_i (curve c). S1 concentration was 1.5 mg/mL. Conditions: 30 mM Hepes, pH 7.3, 1 mM $MgCl_2$. Heating rate, 1 K/min. The vertical bar corresponds to 50 kJ/K·mol.

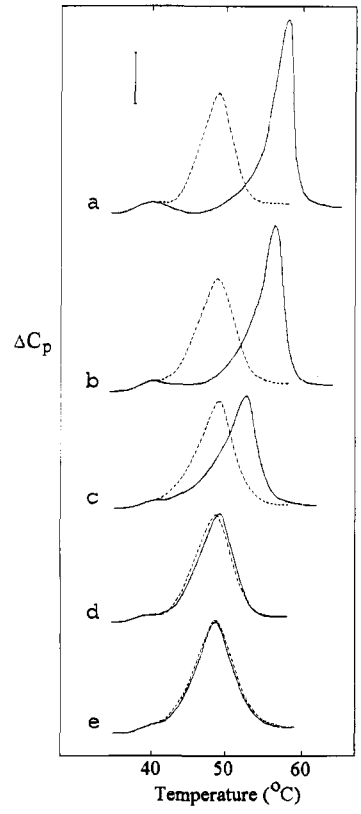

FIGURE 2: Temperature dependencies of excess heat capacity for S1 in the S1-NDP-V_i complexes. Curves shown by straight lines were obtained for S1 in the presence of 0.2 mM V_i and 0.2 mM ADP (curve a), 0.2 mM CDP (curve b), 0.2 mM UDP (curve c), 0.2 mM IDP (curve d), or 0.2 mM GDP (curve e). Curves shown by dotted lines were obtained for S1-NDP complexes in the absence of V_i. Conditions were the same as in Figure 1. Vertical bar corresponds to 100 kJ/K·mol.

Moreover, the same effect has been observed in the case of the binding of PP_i to S1 (Figure 1, curve c). These data lead to the suggestion that the alteration of S1 structure is caused mainly by the binding of the polyphosphate moiety of NDP.

On the other hand, in the presence of vanadate or beryllium fluoride the effects of different NDP strongly depend on the

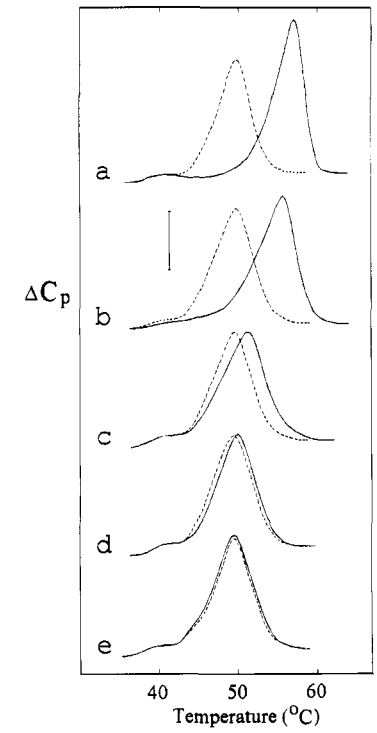

FIGURE 3: Temperature dependencies of excess heat capacity for S1 in the S1-NDP-BeF_x complexes. Curves shown by straight lines were obtained for S1 in the presence of 5 mM NaF, 0.2 mM $BeCl_2$, and 0.2 mM ADP (curve a), 0.2 mM CDP (curve b), 0.2 mM UDP (curve c), 0.2 mM IDP (curve d), or 0.2 mM GDP (curve e). Curves shown by dotted lines were obtained for S1-NDP complexes in the absence of NaF and $BeCl_2$. Conditions were the same as in Figure 1. The vertical bar corresponds to 100 kJ/K·mol.

base structure of NDP. The formation of the S1-ADP-V_i complex results in a major change of S1 conformation which is reflected in a significant shift, by about 9 °C, of the thermal transition to higher temperatures and in an increase in its cooperativity (Figure 2, curve a) (Levitsky et al., 1992). Very similar effect are observed in the case of CDP (Figure 2, curve b). The addition of vanadate to S1 in the presence of UDP results in a significant increase of the S1 thermal stability (the maximum of the transition shifts by about 4 °C to higher temperature); however, the cooperativity of this transition virtually does not change (Figure 2, curve c). A less pronounced effect was observed after addition of vanadate to S1 in the presence of IDP (Figure 2, curve d). Finally, in the presence of GDP the addition of vanadate does not affect the S1 thermal denaturation at all (Figure 2, curve e). Thus, the ability of different NDP to change the S1 thermal denaturation in the presence of V_i decreases in the following order: ADP > CDP ≫ UDP ≫ IDP > GDP. Effectiveness of different NDP in inducing conformational changes in the S1 molecule in the presence of beryllium fluoride follows the same order (Figure 3).

Thermodynamic parameters obtained from the calorimetric measurements on various complexes of S1 with NDP are summarized in Table 1. Analysis of these data shows that the effects of various NDP on the conformation of the S1 molecule are very similar and they do not differ from the effect of PP_i binding to S1. However, in the presence of vanadate or beryllium fluoride the effects of various NDP are quite different from each other. ADP is the strongest affector; and the effectiveness of NDP decreases in the order: ADP > CDP ≫ UDP ≫ IDP > GDP. The effect of

Table 1: Thermodynamic Parameters Obtained from the DSC Data for S1 and Different S1-NDP Complexes

conditions	T_m (°C)	ΔH_{cal} (kJ/mol)	ΔH_{vH} (kJ/mol)	CR
in the absence of NDP				
	48.6 ± 0.2	1110 ± 80	440 ± 50	0.4
in the presence of 0.2 mM NDP				
ADP	49.2 ± 0.2	1120 ± 80	590 ± 80	0.5
CDP	49.2 ± 0.2	1120 ± 80	560 ± 70	0.5
UDP	49.2 ± 0.2	1120 ± 80	520 ± 70	0.5
IDP	49.4 ± 0.2	1120 ± 80	530 ± 70	0.5
GDP	49.4 ± 0.2	1130 ± 80	580 ± 80	0.5
in the presence of 0.3 mM PP				
	49.4 ± 0.2	1130 ± 80	600 ± 80	0.5
in the presence of 0.2 mM NDP, 0.2 mM V_i				
ADP	58.0 ± 0.2	1330 ± 90	1060 ± 140	0.8
CDP	56.8 ± 0.2	1300 ± 90	910 ± 120	0.7
UDP	53.0 ± 0.2	1250 ± 90	500 ± 60	0.4
IDP	49.8 ± 0.2	1130 ± 80	560 ± 70	0.5
GDP	49.4 ± 0.2	1130 ± 80	560 ± 70	0.5
in the presence of 0.2 mM NDP, 5 mM NaF, 0.2 mM $BeCl_x$				
ADP	56.4 ± 0.2	1280 ± 90	900 ± 120	0.7
CDP	55.4 ± 0.2	1290 ± 90	650 ± 80	0.5
UDP	51.0 ± 0.2	1250 ± 90	500 ± 60	0.4
IDP	49.8 ± 0.2	1130 ± 80	560 ± 70	0.5
GDP	49.4 ± 0.2	1130 ± 80	560 ± 70	0.5

last member of this series, GDP, is insensitive to vanadate or beryllium fluoride additions.

It is noteworthy that the cooperativity ratio (CR = $\Delta H_{vH}/\Delta H_{cal}$) decreases in the same order. A value of CR below unity suggests the presence of domains, and the increase of CR indicates an increased cooperativity of the structure (Bertazzon & Tsong, 1990). This is consistent with our recently published data on the S1 domain structure. After decomposition of the total heat sorption curve of S1 into elementary peaks corresponding to melting of separate cooperative regions (domains), three such domains were revealed in the S1 molecule (Levitsky et al., 1990, 1992). However, only two domains were observed in the S1 complexes, S1-ADP-V_i (Levitsky et al., 1992) and S1-ADP-BeF_x (Bobkov et al., 1993). It was concluded that the domain structure of S1 in the complexes S1-ADP-V_i and S1-ADP-BeF_x is similar and yet quite different from the domain structure of nucleotide-free S1 and S1 in the S1-ADP complex (Bobkov et al., 1993). Thus, a significant increase of the CR value in the case of the S1-ADP-V_i and S1-ADP-BeF_x complexes (Table 1) reflects global conformational changes in the S1 molecule which are accompanied by the change of S1 domain structure. The increase of the CR value was also observed for the formation of the S1-CDP-V_i complex. In all other cases including S1-CDP-BeF_x complex as well as S1-NDP-V_i and S1-NDP-BeF_x complexes with UDP and IDP, the differences in CR values did not exceed the errors of CR determination (±0.1) (Table 1). Apparently, the S1 domain structure is not altered much in these complexes.

The lack of any changes after addition of vanadate or beryllium fluoride to S1-GDP indicates that the complexes S1-GDP-V_i and S1-GDP-BeF_x are not formed. In this case, as well as for all S1-NDP complexes, the binding of NDP (or PP_i) to S1 causes only local conformational changes in the S1 molecule which are reflected in a slight but reliable increase of the CR value (Table 1). Such local changes are not accompanied by significant changes of S1 domain structure (Levitsky et al., 1992).

Another parameter which is usually used for the characterization of S1-ADP-V_i and S1-ADP-BeF_x complexes is the stability of these complexes. The complexes decompose slowly after removal of excess reagents, and usually this process is reported by recovery of the S1 ATPase activity (Werber et al., 1992; Phan & Reisler, 1992). The half-lives of the stable complexes at 0 °C, estimated by this approach, were 4 days for the S1-ADP-V_i and 7 days for the S1-ADP-BeF_x (Werber et al., 1992). However, this approach could not be used for S1 complexes with NDP other than ADP and CDP, since they showed appreciable K^+-EDTA-ATPase activity even in the presence of vanadate or beryllium fluoride, before removal of excess reagents (about 20−25% of the activity of control S1 for the complexes with UDP and IDP, and more than 75% in the case of GDP). The dilution of S1 preparations with EDTA-containing buffer and the subsequent addition of ATP, in the course of K^+-EDTA-ATPase measurements, may accelerate by itself the decomposition of these complexes. Therefore, we used the DSC method for monitoring the stability of these complexes. We investigated the S1 thermal denaturation after removal of excess reagents from the S1-NDP-V_i and S1-NDP-BeF_x complexes. The complexes decomposition was linked to the disappearance of the peak attributed to the complex and the corresponding appearance of the peak assigned to nucleotide-free S1. However, this approach can be used only for the characterization of the stability of those complexes whose calorimetric peaks are clearly distinguishable from the peak of nucleotide-free S1 on the thermogram. The complexes with ADP, CDP, and UDP (Figures 2 and 3, curves a−c) meet these demands.

Figure 4 shows results of a decomposition of the S1-ADP-V_i, S1-CDP-V_i, and S1-UDP-V_i complexes. The complexes were dialysed for 2 days against 1 mM $MgCl_2$, and 30 mM Hepes, pH 7.3, at 4 °C to remove the free NDP and V_i and then subjected to calorimetric measurements. It is clear from Figure 4 that the removal of the reagents causes the appearance of the heat sorption peak with a maximum at about 48 °C which is characteristic of the nucleotide-free S1 and a decrease of the peak for the S1-NDP-V_i complex. This effect was negligible for the S1-ADP-V_i complex (Figure 4a) and more pronounced for the S1-CDP-V_i complex (Figure 4b). On the other hand, the complex S1-UDP-V_i

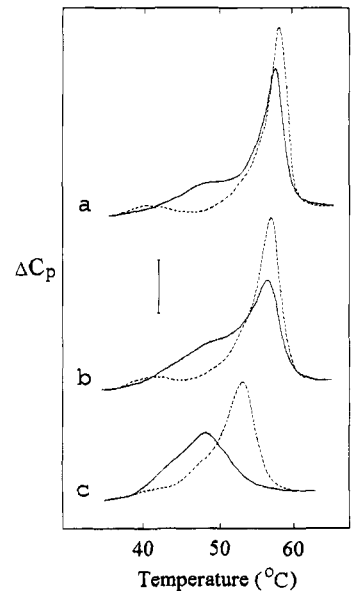

FIGURE 4: Temperature dependencies of excess heat capacity for S1 in the complexes S1-ADP-V_i (a), S1-CDP-V_i (b), and S1-UDP-V_i (c) before and after removal of excess reagents. Curves shown by dotted lines were obtained for S1 in the presence of 0.2 mM NDP and 0.2 mM V_i. Curves shown by straight lines were obtained after removal of excess NDP and V_i from the S1-NDP-V_i complexes by dialysis against 30 mM Hepes, pH 7.3, containing 1 mM $MgCl_2$, at 4 °C for 2 days. Conditions were the same as in Figure 1. The vertical bar corresponds to 100 kJ/K·mol.

was completely decomposed since in this case only the peak which is characteristic of the nucleotide-free S1 was observed (Figure 4,c). This means that the complex S1-UDP-V_i is less stable than the S1-ADP-V_i and S1-CDP-V_i complexes. The stability of the S1-NDP-V_i complexes decreases in the following order: ADP > CDP > UDP. A similar order was found for the S1 complexes with NDP and BeF_x (data not shown). Thus, the stability of the complexes correlates well with the ability of NDP to induce conformational changes in the S1 molecule in the presence of V_i or BeF_x.

This correlation leads to the suggestion that a weakened effect of UDP on S1 conformation in the complexes S1-UDP-V_i and S1-UDP-BeF_x (Figures 2 and 3, curves c) might be caused by a low affinity of UDP in these complexes for the S1 active site. In such a case the thermal denaturation of S1 in the presence of UDP and V_i or BeF_x should strongly depends on the concentration of UDP added. In order to check such a possibility, we have examined the S1 thermal denaturation in the presence of 0.2 mM V_i and different concentrations of UDP (from 30 to 600 μM). Over this range of UDP concentrations the S1-UDP-V_i complex heat sorption curve did not practically differ from that obtained for this complex in the presence of 0.2 mM UDP (Figure 2, curve c). This means that the S1 was saturated with UDP and V_i. On the basis of these data, it may be concluded that the different effects of various NDP on the S1 conformation in the complexes S1-NDP-V_i and S1-NDP-BeF_x do not directly depend on different affinity of NDP for the S1 active site.

DISCUSSION

The results of this work lead to the conclusion that the ability of different NDP to induce conformational changes of S1, in the S1-NDP-V_i and S1-NDP-BeF_x complexes, strongly depends on the NDP base structure. Their effectiveness in inducing such changes decreases in the following order: ADP > CDP ≫ UDP ≫ IDP > GDP. ADP is the most effective while GDP is unable to induce the global conformational changes in the S1 molecule in the presence of V_i or BeF_x. A very similar order of NDP effects was deduced by Hiratsuka (1984), who studied the fluorescence emission and acrylamide quenching of ribose-modified fluorescent analogues of NDP trapped by V_i in the active site of the S1 ATPase. Thus, the author showed that the hydrophobicity of the environment around the fluorophore and the degree of its burial in the protein vary with the base structure of NDP. It was found in that work that the fluorophore of the ADP analogue is the most buried into the protein, while that of the GDP analogue is the least buried one (Hiratsuka, 1984). The comparison of these data with our present results leads to the conclusion that the effectiveness of different NDP in inducing the global conformational changes of the S1 in the S1-NDP-V_i and S1-NDP-BeF_x complexes depends on the degree of their burial in the protein, which in turn is determined by the base structure of NDP.

The effects of ADP and CDP complexes with V_i and BeF_x on S1 are rather similar. However, they differ radically from the effects of all other NDP studied (Figures 2−4 and Table 1). Thus, the 6-amino group of the adenine moiety (and corresponding 4-amino group of the cytidine) seem to be essential for inducing conformational changes in S1 via formation of S1-NDP-V_i and S1-NDP-BeF_x complexes. Specific interactions of these amino groups, hydrogen-bond donors, with appropriate groups of the protein seem to play an important role in the structural transformation of the S1 molecule. On the other hand, UDP, IDP, and GDP in which the amino position is occupied by a carbonyl, a hydrogen-bond acceptor, seem to be unable to form such interactions, and their effects on the S1 conformation are smaller. A group at the C2 position is also very important since GDP, which has a bulky amino group at this position, is unable to induce any conformational changes of the S1 molecule in the presence of V_i or BeF_x (Figures 2 and 3 and Table 1).

The order of NDP effectiveness in inducing conformational changes of S1 in the S1-NDP-V_i and S1-NDP-BeF_x complexes, ADP > CDP ≫ UDP ≫ IDP > GDP, is very similar to that found for kinetic parameters of the myosin NTPase (Kielley et al., 1956; Blum, 1955; 1960; Seidel, 1975) and to the order of NTP which induce enhanced tryptophan fluorescence in heavy meromyosin (Werber et al., 1972). Since the enhanced intrinsic fluorescence reflects the formation of the predominant intermediate M**-NDP-P_i during NTPase reaction, one can conclude that conformational changes of S1 induced by the formation of the S1-NDP-V_i and S1-NDP-BeF_x complexes correspond to the structural changes which occur in the myosin head due to the formation of the M**-NDP-P_i intermediate during NTPase reaction. The lack of any changes after addition of V_i or BeF_x to S1-GDP (Figures 2 and 3, curve e; Table 1) correlates with the data of Eccleston and Trentham (1979) that this long-lived intermediate is not formed during GTP hydrolysis.

It is noteworthy that the same order of effectiveness was recently found in studies on the effect of different NTP on isometric force and unloaded shortening velocity in rabbit glycerinated muscle fibers (Regnier et al., 1993; Pate et al., 1993) and on thin filament movement in the motility assays (Homsher et al., 1993). In the study of kinetics of the NTP

hydrolisis by acto-S1 the maximum rate of steady state hydrolysis for ATP was close to those for CTP but significantly bigger than for GDP (White et al., 1993). This correlation indicates that structural changes shown above play a very important role in the energy transduction process during ATP-dependent cyclic interactions of myosin heads with actin.

It is important to note that in the absence of V_i or BeF_x the effects of various NDP and PP_i on the S1 structure do not differ from each other. This suggests that adenine and ribose moieties do not play an important role in this effect. We propose the following explanation. ADP, probably, binds to the S1 active site mainly via its polyphosphate moiety whereas adenine and ribose do not participate strongly in this process. In this case adenine moiety of ADP is not buried deeply in the active site, and its 6-amino group does not interact with appropriate groups on the protein. Consequently, the interaction of ADP and all other NDP with S1 cause only local conformational changes in the S1 molecule, probably near the polyphosphate-binding site. However, the manner of ADP binding is drastically altered after formation of the S1-ADP-V_i and S1-ADP-BeF_x complexes. In this case the deep burial of the adenine moiety into the S1 active site occurs, and 6-amino group interacts specifically with the protein groups. These interactions lead to the global conformational change of the S1 molecule. Probably, similar changes are caused by ATP binding to S1, since the domain structure of S1 in the complex with nonhydrolyzable ATP analogue, AMP-PNP, was shown to be similar to that of S1 in the S1-ADP-V_i complex (Levitsky et al., 1992). Thus, the occupancy of phosphate sites either by three phosphates in the M**-ATP and M**-ADP-P_i complexes or by two phosphates of ADP and V_i or BeF_x seems to be essential for deep burial of the nucleotide into the myosin active site and for inducing the global conformational changes in the myosin head.

In summary, DSC studies on the S1-NDP-V_i and S1-NDP-BeF_x complexes offer a new and promising approach to the investigation of the structural changes which occur in the myosin head due to the formation of M**-NDP-P_i intermediates during NTP hydrolysis.

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