Differential scanning calorimetric study of the thermal unfolding of the motor domain fragments of Dictyostelium discoideum myosin II

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The thermal unfolding of two recombinant fragments of the head of Dictyostelium discoideum myosin II was studied by differential scanning calorimetry. These fragments M754 and M761 correspond to the globular motor portion of the myosin head that contains ATP- and actin-binding sites but lacks the light chain binding domain. Our results show that M754 is less thermostable than M761: the maximum of the thermal transition occurred at 41.7°C for M754 and at 45.6°C for M761, and the calorimetric enthalpy value determined for M754 (677 kJ/mol) was about half of that for M761 (1417 kJ/mol). This indicates that the region containing residues 755–761 plays a very important role in the structural stabilization of the entire globular motor part of the myosin head. ADP binding induces structural changes in both myosin fragments which are reflected in a 2–3.5°C shift of the thermal transitions to higher temperature. The formation of stable ternary complexes of these myosin fragments with ADP and phosphate analogues such as orthovanadate, beryllium fluoride or aluminium fluoride causes additional structural changes which are reflected in a pronounced increase of thermal stability. The effect of beryllium fluoride was less distinct than that of aluminium fluoride or orthovanadate. In general, the changes caused by various phosphate analogues were similar to those observed with skeletal myosin subfragment 1. Thus, structural changes revealed by differential scanning calorimetry in the myosin head, that are due to the formation of stable ternary complexes with ADP and P, analogues, occur mainly in the globular motor portion of the head.

Keywords: myosin head; thermal unfolding; differential scanning calorimetry; Dictyostelium discoideum.

Members of the myosin family convert chemical energy to force and displacement by interaction with ATP and actin filaments. This interaction is the basis of muscle contraction and a number of events in cell motility. The model of the atomic structure of chicken skeletal S1 revealed that the myosin head consists of a globular motor domain that contains both the catalytic and actin-binding sites, and a neck region which consists of a 8.5-nm α-helix that emerges from the globular part of the heavy chain and is stabilized by the binding of the essential and regulatory light chains (Raymont et al., 1993).

In recent years, genetically truncated myosin head fragments obtained from the lower eukaryote Dictyostelium discoideum have been successfully used for resolving the structure and properties of the myosin head. Such fragments truncated at amino acid residues 754–765 correspond to the globular motor portion of the myosin head that contains ATP- and actin-binding sites but lacks the neck region and light chains. These fragments were shown to retain the ability to bind and hydrolyze ATP, to bind actin (Kurzawa et al., 1997; Bobkov et al., 1997), and to move actin filaments when connected to an artificial lever arm (Itakura et al., 1993; Anson et al., 1996). The fragment genetically truncated at residue 759 (S1Dc) was used to resolve the atomic structure of the motor domain of the myosin head complexed with MgADP and P, analogues, such as orthovanadate (V−), beryllium fluoride (BeF−), or aluminium fluoride (AlF−) (Fisher et al., 1995a, b; Smith and Raymont, 1996). An intriguing result of these studies was that the structure of S1Dc · ADP · BeF− was remarkably similar to that of the equivalent portion of nucleotide-free chicken skeletal S1, while the structures of S1Dc · ADP · AlF− and S1Dc · ADP · V− exhibited significant changes. Based on these results, the authors postulated that S1Dc · ADP · BeF− corresponds to a pre-hydrolysis state (S1*D · ATP) while the complexes S1Dc · ADP · AlF− and S1Dc · ADP · V− mimic the main transition state S1** · ADP · P, for the hydrolysis of ATP (Fisher et al., 1995a, b; Smith and Raymont, 1996).

In contrast, previous solution studies performed with skeletal S1 showed that the formation of the complexes S1 · ADP · Vi, S1 · ADP · AlF− and S1 · ADP · BeF− cause significant conformational changes in the S1 molecule (Werber et al., 1992; Phan and Reisler, 1992). A global change of the whole S1 molecule

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Abbreviations: DSC, differential scanning calorimetry; S1, myosin subfragment 1; S1Dc, M761 and M754, myosin head fragments of Dictyostelium discoideum myosin II containing 759, 761 and 754 amino-acid residues, respectively; V−, orthovanadate; BeF−, beryllium fluoride; AlF−, aluminium fluoride; LCBD, light-chain-binding domain; ΔHm, transition temperature; ΔSm, calorimetric enthalpy, (the area under the heat sorption curve); Δts, the width at the half-height of the thermal transition.

Enzyme. Myosin ATPase (EC 3.6.1.32).
caused by the formation of these complexes was also indicated from significant changes in the shape, radius of gyration, and hydrophobicity of S1, as revealed by several other techniques (Highsmith and Eden, 1990; Sugimoto et al., 1995; Gopal and Burke, 1996). In particular, the thermal unfolding of S1 in the S1·ADP·V and S1·ADP·BeF complexes was studied by means of differential scanning calorimetry (DSC) (Levitsky et al., 1992; Bobkov et al., 1993; Bobkov and Levitsky, 1995).

DSC is the most effective and commonly employed method to study the thermal unfolding of proteins (Privalkov and Potekhin, 1986; Shnyrov et al., 1997b). Information regarding the presence of domains, and the effects of substrate binding on domain stability and interactions, can be determined with this method (Privalkov, 1982; Sturtevant, 1987; Brands et al., 1989). It has been shown by using DSC that the formation of both S1·ADP·V and S1·ADP·BeF complexes causes a similar change of S1 conformation which is reflected in a pronounced increase of S1 thermal stability and in a significant change of S1 domain structure (Levitsky et al., 1992; Bobkov et al., 1993; Bobkov and Levitsky, 1995). These results are in good agreement with the data obtained by other methods which suggest that the structure of S1 is similar in the complexes S1·ADP·V, S1·ADP·AlF4- and S1·ADP·BeF (Sugimoto et al., 1995; Gopal and Burke, 1996). A significant difference between S1·ADP·V and S1·ADP·BeF complexes has been revealed by DSC studies only when S1 was reductively methylated or modified at residue Cys70/73 or Lys83 (Golitsina et al., 1996).

Thus, the DSC studies on skeletal S1 revealed significant structural changes on forming both S1·ADP·V and S1·ADP·BeF complexes which is in contradiction with the data of crystallographic analysis which do not show any significant structural changes in forming the S1Dc·ADP·BeF complex. However, these data were obtained by different methods and with different proteins. To clarify the difference between DSC and crystallographic results, it is necessary to compare the properties of proteins by the same method. Therefore in the present study we used DSC to examine M761, a genetically truncated fragment of D. discoideum myosin II. This fragment represents, like S1Dc, the isolated motor domain of the myosin head, and its transient kinetic and actin motility properties have been recently characterized (Kurzawa et al., 1997; Anson et al., 1996).

Another goal of this study was to use DSC to compare the thermal unfolding of M761 with that of a D. discoideum myosin head truncated at Ile754 (M754). The properties of M761 were shown to be almost identical to that of D. discoideum full-length S1-like fragment M864 in regard to nucleotide binding, nucleotide hydrolysis, actin binding and the interaction between actin and nucleotide binding sites (Kurzawa et al., 1997; Ritchie et al., 1993). In contrast, truncation or alterations of the heavy chain at or near Ile754 dramatically affect the nucleotide-binding site and the communication between the nucleotide and actin-binding sites (Woodward et al., 1995; Kurzawa et al., 1997). One explanation for the behaviour of M754 compared to M761 or M864 was that the smaller fragment is less stable but that it can be stabilised by either actin or nucleotide binding.

MATERIALS AND METHODS

Protein expression and purification. D. discoideum transformants were grown at 21°C in DD broth 20 containing (per litre): 20 g protease peptone L85 (Oxoid), 7 g yeast extract (Oxoid), 8 g glucose, 0.33 g NaHPO4, 7H2O, and 0.35 g KH2PO4. Transformants were obtained by electroporation and continuously grown in the presence of 10 μM/ml of the aminoglycoside G418 (Manstein et al., 1995). Plasmids used for transformation were derivatives of the extrachromosomal vectors pDXA-3H and pDXA-HC (Manstein et al., 1995). M754 and M761 carry a C-terminal affinity tag consisting of eight His residues. Transformants were screened for protein production and the His-tagged myosin fragments were purified as described by Manstein and Hunt (1995). Yields of up to 8 mg of each construct/culture medium were obtained and SDS gels showed the presence of a single band of protein when loaded at 10 μg/lane.

Preparation of the complexes of M761 or M754 with ADP and P, analogues. Trapping of ADP by different phosphate analogues (V, BeF3-, AlF6-3-) was performed by the methods described for the preparation of stable ternary complexes S1·ADP·V, S1·ADP·AlF6- and S1·ADP·BeF3- (Goodno, 1982; Werber et al., 1992; Bobkov and Levitsky, 1995). To obtain these complexes, M761 or M754 (0.75 mg/ml) were incubated with 0.4 mM ADP and 0.4 mM V, BeF3- or AlF6-3- for 30 min at 20°C in a medium containing 30 mM Hepes pH 7.3, 1 mM MgCl2. Fluorometric complexes formed with beryllium and aluminium were obtained by addition of 0.4 mM BeCl2 or AlCl3 in the presence of 5 mM NaF. The formation of the complexes was controlled by measuring the Km-EDTA-ATPase activity of the protein. ATPase activity of M761 or M754 modified by V, BeF3- or AlF6-3- in the presence of ADP did not exceed 3–5% of the activity of unmodified protein preparation.

Differential scanning calorimetry (DSC). DSC experiments were performed on a DASM-4 M differential scanning microcalorimeter (Institute for Biological Instrumentation, Pushchino, Russia) with cell volumes of 0.48 ml, at a scanning rate of 1 K/min, interfaced with a personal computer (IBM compatible). Prior to DSC experiments, the proteins were dialyzed against 30 mM Hepes pH 7.3, 1 mM MgCl2. Before the measurements, samples were degassed by stirring in an evacuated chamber for 10 min at room temperature and then immediately loaded into the calorimeter cell. The final dialysis buffer (also degassed) was loaded into the reference cell. A pressure of 152 kPa (1.5 atm) of dry nitrogen was always kept over the liquids in the cells throughout the scans to prevent any degassing during heating. The reversibility of the thermal transitions was checked by a second heating of the sample immediately after cooling from the first scan. The calorimetric traces were corrected for the instrumental background by subtracting a scan with buffer in both cells. The enthalpy data was corrected using a chemical baseline as suggested by Takahashi and Sturtevant (1981). The temperature dependence of the molar heat capacity was further analyzed and plotted using the Windows-based software package Origin (MicroCal Inc., Northampton MA, USA). Calorimetric enthalpy of the transitions, ΔHcal, was determined from the areas under the heat-capacity curves by means of a 25-μW electric calibration mark.

Most measurements were performed at a protein concentration of 0.7–1.0 mg/ml. The denaturation of both proteins (M761 and M754) studied as well as of skeletal myosin S1 were found to be fully irreversible, as no thermal effect was observed in a second heating of the protein solutions.

Molecular masses of 89.2 kDa, 88.2 kDa, and 115 kDa were used for M761, M754, and skeletal S1, respectively.

RESULTS

Comparison between myosin head fragments M761 and M754. Fig. 1 shows calorimetric traces for the thermally induced unfolding of M761 and M754 in the absence of nucleotides. The main calorimetric parameters extracted from this data are the transition temperature, Tm, calorimetric enthalpy, ΔHcal (the area under the heat sorption curve), and the width at the
Fig. 1. DSC scans of M754 and M761 in the absence of nucleotides. Protein concentrations were 0.9 mg/ml. Conditions: 30 mM Hepes pH 7.3, 1 mM MgCl₂. Heating rate was 1 K/min. The parameters derived from this data are shown in Table 1.

Table 1. Calorimetric parameters obtained from the DSC data for M761 and M754, in comparison with those for skeletal myosin S1.

The absolute error of the given \( t_m \) values did not exceed ±0.2°C. The relative error of the given \( \Delta H_{ad} \) values did not exceed ±6%. \( \Delta t_{0.5} \), the width at the half-height of the thermal transition, is a relative measure for the cooperativity of the transition. Experimental conditions for all measurements were 30 mM Hepes pH 7.3, 1 mM MgCl₂.

<table>
<thead>
<tr>
<th>Fragment or complex</th>
<th>( t_m ) (°C)</th>
<th>( \Delta H_{ad} ) (kJ·mol⁻¹)</th>
<th>( \Delta t_{0.5} ) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M761 alone</td>
<td>45.6</td>
<td>1417</td>
<td>4.9</td>
</tr>
<tr>
<td>M761 · ADP</td>
<td>49.1</td>
<td>1400</td>
<td>4.3</td>
</tr>
<tr>
<td>M761 · ADP · BeF₆⁻</td>
<td>52.7</td>
<td>1572</td>
<td>3.7</td>
</tr>
<tr>
<td>M761 · ADP · AlF₆⁻</td>
<td>54.9</td>
<td>1772</td>
<td>3.8</td>
</tr>
<tr>
<td>M761 · ADP · V₅</td>
<td>55.4</td>
<td>1588</td>
<td>3.8</td>
</tr>
<tr>
<td>M754 alone</td>
<td>41.7</td>
<td>677</td>
<td>4.8</td>
</tr>
<tr>
<td>M754 · ADP</td>
<td>43.9</td>
<td>740</td>
<td>4.2</td>
</tr>
<tr>
<td>M754 · ADP · BeF₆⁻</td>
<td>47.1</td>
<td>949</td>
<td>4.7</td>
</tr>
<tr>
<td>skeletal S1 alone</td>
<td>49.5</td>
<td>1505</td>
<td>7.2</td>
</tr>
<tr>
<td>S1 · ADP</td>
<td>50.6</td>
<td>1618</td>
<td>5.5</td>
</tr>
<tr>
<td>S1 · ADP · BeF₆⁻</td>
<td>57.3</td>
<td>1726</td>
<td>3.9</td>
</tr>
<tr>
<td>S1 · ADP · AlF₆⁻</td>
<td>58.4</td>
<td>2002</td>
<td>3.2</td>
</tr>
<tr>
<td>S1 · ADP · V₅</td>
<td>58.9</td>
<td>1805</td>
<td>3.0</td>
</tr>
</tbody>
</table>

half-height of the transition, \( \Delta t_{0.5} \), which is a relative measure for the cooperativity of the transition. These are summarized in Table 1 and compared with those obtained earlier on skeletal myosin S1 (Bobbkov and Levitsky, 1995; Levitsky et al., 1995). Under these conditions, M754 is clearly less thermostable than M761: The maximum of the thermal transition is at 41.7°C for M754 and at 45.6°C for M761, and the value of calorimetric enthalpy \( \Delta H_{ad} \) determined for M754 (677 kJ/mol) is about half of that for M761 (1417 kJ/mol) (Fig. 1, Table 1).

Both fragments represent the isolated motor part of the myosin head but M754 differs from M761 by the absence of myosin heavy chain residues 755–761. Thus the data suggests that this region plays a very important role in the structural stabilization of the entire motor head of the myosin head.

Fig. 2. DSC scans of M761 (A) and M754 (B) in the presence of 0.4 mM ADP. Curves shown by dashed lines were obtained in the absence of ADP. Conditions were the same as in Fig. 1.

Fig. 3. DSC scans of M761 in the presence of ADP and BeF₆⁻, AlF₆⁻ or V₅. Ligand concentrations 0.4 mM ADP (control curve in dotted line), with 5 mM NaF and 0.4 mM BeCl₂ (BeF₆⁻), 5 mM NaF and 0.4 mM AlCl₃ (AlF₆⁻), or 0.4 mM V₅ (V₅). Other conditions were the same as in Fig. 1 except chemical baselines were subtracted from the experimental curves.

Effects of ADP binding to M761 and M754. Previous DSC studies of S1 from rabbit skeletal muscle myosin have shown that the binding of ADP to S1 does not significantly affect the temperature of the thermal transition, but it increases the cooperativity of the transition (Bobbkov and Levitsky, 1995). In contrast, ADP binding to M761 or M754 induced a pronounced shift of the thermal transition to higher temperature (Fig. 2). This ADP-induced shift was more pronounced for M761 (3.5°C) than that for M754 (2.2°C). The binding of ADP to M761 or M754 also increased the cooperativity of the thermal transition: \( \Delta t_{0.5} \), decreased in the presence of ADP from 4.9°C to 4.3°C for M761, and from 4.8°C to 4.2°C for M754. In this respect M761 and M754 are rather similar to each other and to skeletal S1 (Table 1).

Fig. 4. DSC scans of M754 in the presence of ADP and phosphate analogues. Previous DSC studies performed with skeletal S1 have shown that DSC is useful for probing the global conformational changes of the S1 molecule caused by ligand binding. The formation of stable ternary complexes of S1 with ADP and P, analogues such as V₅, BeF₆⁻, AlF₆⁻ caused a significant increase of S1 thermal stability \( (t_m, \Delta H_{ad}) \), and a considerable increase in the cooperativity \( (\Delta t_{0.5}) \) of the thermal transition (Bobbkov and Levitsky, 1995; Levitsky et al., 1995). Figure 3
show the effects of the formation of the ternary complexes of M761 with ADP and various P, analogues, in comparison with the effect of ADP alone (control curve shown by dotted line). In all cases the formation of the ternary complexes causes a significant shift of the thermal transition to higher temperature. The effects of V, and AlF$_4^-$ were almost the same, whereas the effect of BeF$_2$ was less pronounced (Fig. 3). Similar results were obtained for the ternary complexes formed with M754. Figure 4 shows how binding of ADP and BeF$_2$ causes a significant increase of the thermal stability of the protein (Fig. 4).

Analysis of these data shows a strong similarity between M761 and S1 in formation of the ternary complexes with ADP and P, analogues. In both cases the most pronounced structural changes were observed with V, and AlF$_4^-$, These changes were reflected in increased $\Delta t_n$ and $\Delta H_{\text{ad}}$ values, and in a decrease of the $\Delta A_{t_n}$ value, indicative of the increased cooperativity of the thermal transition (Table 1). In the case of the ternary complex with BeF$_2$, the cooperativity was similar to that of the AlF$_4^-$ and V, complexes whereas $t_n$ and $\Delta H_{\text{ad}}$ were midway between the values for ADP alone and with V, For S1 all values were much closer to those seen with AlF$_4^-$ or V, than to ADP alone.

Thus, in spite of the fact that M761 is devoid of the light-chain-binding domain and light chains it is able, like skeletal myosin S1, to undergo global structural changes in forming the stable ternary complexes with ADP and P, analogues. This suggests that these structural changes occur mainly in the globular motor part of the head.

**DISCUSSION**

**Comparison of M761 with skeletal S1.** The data presented here show that the *D. discoideum* myosin head fragment M761 is similar to rabbit skeletal S1 whose properties were described in previous studies (Levitsky et al., 1992, 1995; Bobkov and Levitsky, 1995). However, there are some notable differences between M761 and S1.

M761 is less thermostable than rabbit skeletal S1 with a 4°C lower $t_m$. Thermal inactivation of the Ca$^{2+}$-ATPase of S1Dc occurred at 35°C also indicating lower thermal stability of the *Dictyostelium* protein (Bobkov et al., 1997). A 5°C lower $t_m$ for the myosin head of *Acanthamoeba castellanii* myosin II compared to rabbit skeletal myosin was reported in comparative DSC studies (Zolkiewski et al. 1995). Thus, the thermal stability of the myosin head may be a specific feature of the source of myosin and the difference in thermal stability between M761 and S1 may not be related to the loss of the light-chain-binding domain. This suggestion is supported by the data showing that the kinetic properties of M761 are almost identical to that of full-length myosin head of *D. discoideum* myosin II (Kurzawa et al., 1997).

M761 denatures more cooperatively than S1. Similar enthalpy values were measured for S1 and M761 but the $\Delta A_{t_n}$ values were 7.2°C and 4.9°C, respectively (Table 1). This difference could be explained by different calorimetric domain structures of S1 and M761. In previous studies the domain structure of S1 was elucidated from DSC data using the successive annealing procedure (Shnyrov et al., 1984; 1997a; Levitsky et al., 1992) which is applied to fully or partially irreversible thermal transitions. This decomposes experimentally the total heat sorption curve of S1 into elementary peaks corresponding to melting of separate cooperative regions or calorimetric domains and three such domains were observed for S1 (Levitsky et al., 1990, 1992). Three domains (the peaks 1, 2, and 3 with maxima at 44, 49, and 51.5°C, respectively) were also identified after computer deconvolution of the heat sorption curve using a non-two-state model (Fig. 5A). The computer deconvolution of heat sorption curves into domain structures is a controversial method and the following discussion has to be accepted in this light. However, in the case of S1, two independent methods of deconvolution predicted three structural domains which gives some confidence in the predictions. Levitsky (1994) suggested that calorimetric domain 3 corresponded to the LCBD and Golitsina et al. (1992) presented evidence that domain 1 contained approximately equal contributions from the melting of the alkali light chain and a globular part of the heavy chain.

In contrast to S1, only two calorimetric domains were observed for M761 (peaks 1 and 2 with maxima at 43.5 and 45.8°C) as shown in Fig. 5B. The third and most thermostable domain of S1 is absent (Fig. 5B). The presence of only two calorimetric domains in M761, has been recently obtained by using the successive annealing procedure (M. A. Ponomarev, personal communication). This supports the assignment of domain 3 to the LCBD. The narrower overall thermal transition for M761 compared to S1 is thus seen to be simply due to loss of the most thermostable domain.

The effect of ADP on thermal unfolding. ADP binding to M761 and S1 increases the cooperativity of the transition without affecting its enthalpy. $t_m$ for M761 shifts from 45.6°C to 49.1°C, whereas $t_m$ changes only by +1.1°C for S1 (Fig. 2A, Table 1). This difference can be explained by the way in which the individual calorimetric domains respond to ADP binding as shown in Fig. 5. In this interpretation, M761 and S1 are very similar in their responses to ADP binding with both calorimetric domains of the globular motor part increasing in thermal stability whereas the contributions from the alkali light chain (half of calorimetric domain 1) and the LCBD (calorimetric domain 3) are unaffected by ADP binding.

**Structural changes in M761 and M754 complexed with P, analogues.** Addition of ADP and P, analogues to M761 induces a 7–10°C increase in the thermal stability, as in the case of S1 (Table 1). The results of this work show that M761 undergoes structural changes due to the formation of stable ternary complex with ADP and BeF$_2$. These changes result in an elevated temperature of denaturation, increased enthalpy, and a greater cooperativity of the thermal transition and their size is similar to those observed for the ADP - V, and ADP - AlF$_4^-$ complexes (Fig. 3, Table 1). Similar DSC results were observed for S1 (Table 1; Bobkov and Levitsky, 1995; Levitsky et al., 1995) and the structural change on forming the S1 - ADP - V, S1 - ADP -
Fig. 5. Deconvolution of thermograms of rabbit skeletal myosin S1 (A, C) and D. discoideum myosin head fragment M761 (B, D) in the absence of nucleotide (A, B) and in the presence of 0.4 mM ADP (C, D). Solid lines represent the experimental curves after subtraction of chemical baselines, the dashed lines resulted from fitting the data to the non-two-state model and dotted lines represent the elementary transitions (calorimetric domains). Three transitions were obtained for S1 with and without ADP: without ADP, \( t_n \) values were 44, 49 and 51°C, with \( \Delta H_n \), 578, 639 & 295 kJ mol\(^{-1} \); in the presence of ADP, \( t_n \) values were 44.7, 48.6, and 51.1°C with \( \Delta H_n \) 342, 403 & 871 kJ mol\(^{-1} \). Two transitions were obtained for M761: in the absence of ADP, \( t_n \) values were 43.5 and 45.8°C, with \( \Delta H_n \) 636 and 795 kJ mol\(^{-1} \); with ADP, \( t_n \) values were 46.5 and 49.1°C with \( \Delta H_n \) 588 and 822 kJ mol\(^{-1} \).

\( \text{AlF}_4^- \) and S1 - ADP - BeF\(_2\) complexes revealed have been confirmed by other techniques (Sugimoto et al., 1995; Gopal and Burke, 1996). In terms of the domain structure, the \( t_n \) of both calorimetric domains of M761 increase by 3.5 - 4°C after addition of BeF\(_2\), (Fig. 5D). A very similar effect of BeF\(_2\), was observed for M754, (Fig. 4). As for M761, the total heat sorption curve of nucleotide-free M754 was decomposed into two calorimetric domains with maxima at 39.5°C and 41.9°C. These transitions shifted in the presence of ADP by 2.5 - 3°C to higher temperature with a further shift by 2.5 - 3°C after addition of BeF\(_2\), (data not shown).

Thus, there is clear evidence that the formation of the complex with ADP - BeF\(_2\), does induce structural changes in the motor part of Dictyostelium myosin head, although these changes were not revealed by earlier published crystallographic studies (Fisher et al., 1995a,b). However, the recent crystal structure of M754 complexed with ADP - BeF\(_2\), does appear to be similar to earlier published structures of S1Dc complexed with ADP - V, (L. Schlichting, K. C. Holmes and D. J. Manstein, personal communication). This suggests that the conformation of the protein in the crystal may critically depend upon details of the crystallization conditions and crystal packing.

Comparison of M754 with M761. The effects of ADP and phosphate analogues on M754 were very similar to those on M761 (Fig. 2 - 4, Table 1). However under all conditions M754 is less thermostable than M761.

M754 differs from M761 by the absence of myosin heavy chain residues 755 - 761. The loss of these seven residues has a dramatic effect on the thermal stability of the head fragment, as shown by the significant decrease in \( t_n \) and calorimetric enthalpy of M754 compared to M761 (Fig. 1, Table 1). This observation may help to explain some of the differences in the kinetic behaviour of M754 and M761 (Kurzawa et al., 1997). It seems possible that the region between residues 754 - 761 is very important for stabilization of the entire motor part of the myosin head. Loss of the 755 - 761 peptide does cause a significant destabilization of the head, thus affecting the nucleotide binding site and impairing the communication between the nucleotide and actin-binding sites. In this context it is noteworthy that a very similar destabilization, i.e. a dramatic decrease of the protein thermal stability (about 7°C decrease of \( t_n \) and twofold decrease of calorimetric enthalpy), has been observed for rabbit skeletal S1 after trypic cleavage of its heavy chain between Arg23 and Ile24 (Nikolaeva et al., 1997). This N-terminal cleavage site is located near residues 776 - 782 in the atomic structure of chicken S1 and 755 - 761 of D. discoideum myosin II (Rayment et al., 1993). Comparison of these data suggests that the junction between the motor domain and LCBD, which also serves as a communication pathway between the two domains, is of crucial importance for the structural integrity of the myosin motor.

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