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Calorimetric characterization of the stable complex of myosin subfragment 1 with ADP and beryllium fluoride

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The thermal unfolding of the myosin subfragment 1 (S1) in its stable complex with ADP and beryllium fluoride (S1 · ADP · BeF_3^-) was studied by differential scanning calorimetry. It has been shown that the structure of the S1 molecule in the S1 · ADP · BeF_3^- complex is similar to that of S1 in its complex with ADP and orthovanadate (S1 · ADP · V_i) but differs radically from that of nucleotide-free S1 and S1 in the S1 · ADP complex. It is concluded that the S1 · ADP · BeF_3^- complex can be considered, like the S1 · ADP · V_i complex, a stable structural analogue of the myosin head · ADP · P_i transition state of the myosin-catalyzed ATP hydrolysis.

Myosin subfragment 1; Beryllium fluoride; Scanning microcalorimetry; Rabbit skeletal muscle

1. INTRODUCTION

Muscle contraction and a number of events in cell motility are based on the cyclic interaction of myosin heads with actin which is coupled to myosin-catalyzed ATP hydrolysis. During steady-state ATP hydrolysis a number of discrete intermediates having different spectral properties is formed [1,2]. At room temperature the predominant intermediate is the M** · ADP · P_i complex (M** represents the myosin head having increased intrinsic fluorescence [3]). 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 [2]. The use of stable analogues allows the structure and properties of the myosin head in the M** · ADP · P_i intermediate to be studied. A complex of the isolated myosin head, or myosin subfragment 1 (S1), with ADP and orthovanadate ion (V_i) is often used as stable analogue of the M** · ADP · P_i complex [4,5].

Recently we have studied the thermal denaturation of S1 in the S1 · ADP · V_i complex by means of differential scanning microcalorimetry [6]. It has been shown that the trapping of ADP by V_i in the active site of S1 causes the 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 [6]. Thus, this method allows to register the conformational changes of the whole S1 molecule caused by formation of the S1 · ADP · V_i complex.

A new stable analogue of the intermediate M** · ADP · P_i state has been recently described. This is the complex of S1 with ADP and beryllium fluoride (BeF_3^- [7,8]. This complex is more stable than S1 · ADP · V_i. In contrast to vanadate, beryllium fluoride does not polymerize and it is spectroscopically silent, permitting its usage in fluorescence studies [7,8]. For these reasons S1 · ADP · BeF_3^- is a more attractive analogue of M** · ADP · P_i than S1 · ADP · V_i. However, there are no data on the structure of the S1 molecule in the S1 · ADP · BeF_3^- complex. In the present work we have investigated the thermal denaturation of the S1 · ADP · BeF_3^- complex by means of differential scanning microcalorimetry. The data obtained show that the structure of S1 in this complex is similar to that of S1 in the S1 · ADP · V_i complex is similar to that of S1 in the S1 · ADP · V_i complex.

2. MATERIALS AND METHODS

S1 from rabbit skeletal muscle myosin was prepared by digestion of myosin filaments with chymotrypsin [9]. Concentration of S1 was determined spectrophotometrically by using $E^{1\%}_{280\,nm} = 7.5$. The S1 · ADP · V_i complex was obtained by incubation of S1 (1 mg/ml) with 1 mM $MgCl_2$, 0.2 mM ADP and 0.2 mM V_i [5] for 30 min at 20°C in a medium containing 30 mM HEPES, pH 7.3. In order to obtain the S1 · ADP · BeF_3^- complex, S1 (1 mg/ml) was incubated for 5 min at 20°C in 1 mM $MgCl_2$, 30 mM HEPES, pH 7.3, 0.2 mM ADP, and 5 mM NaF; after addition of 0.2 mM $BeCl_2$ the reaction mixture was further incubated at 20°C for 25 min [7]. The formation of the S1 · ADP · V_i and S1 · ADP · BeF_3^- complexes was controlled by measuring the K^+-EDTA-ATPase activity of S1 [7]. ATPase activity of S1 modified by vanadate or beryllium fluoride in the presence of ADP did not exceed 5% of the unmodified S1 preparation activity.

Calorimetric measurements were carried out on a differential adiabatic scanning microcalorimeter DASM-4 (Russia) as described earlier [6]. All the measurements were carried out in 30 mM HEPES, pH 7.3, containing 1 mM $MgCl_2$ at protein concentration 1 mg/ml and a constant heating rate 1°C/min. Decomposition of the total heat sorp-

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tion curves into elementary peaks was performed by the 'successive annealing' method as described earlier [6]. The denaturation enthalpy, ΔH, was calculated as described by Privalov et al. [10].

3. RESULTS AND DISCUSSION

Fig. 1 shows calorimetric data on the thermally induced unfolding of S1 and of the complexes of S1 with ADP in the absence and in the presence of beryllium fluoride. The binding of ADP to S1 by itself has practically no influence on the temperature of the thermal transition but increases its sharpness (Fig. 1b). Thus, the binding of ADP to S1 causes the 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 ADP the addition of NaF and $BeCl_2$ to S1 does not cause any significant changes in the S1 heat sorption curve (Fig. 1a). On the other hand, the formation of the S1 · ADP · BeF_3^- complex in the presence of ADP, NaF and $BeCl_2$ results in an essential change of S1 conformation which is reflected in the significant shift of the thermal transition and in an increase of its cooperativity (Fig. 1c). This effect is very similar to the effect found earlier for the formation of S1 · ADP · V_i complex [6] (data not shown).

The main thermodynamic parameters of the thermal denaturation (denaturation temperature, T_d, and denaturation enthalpy, ΔH) obtained from the calorimetric measurements on various complexes studied are summarized in Table I. An analysis of these data shows that the S1 · ADP · BeF_3^- and S1 · ADP · V_i complexes are similar and differ radically from S1 · ADP complex and nucleotide-free S1. It is reasonable to assume that the conformation of S1 molecule in the S1 · ADP · BeF_3^- complex is similar to that of S1 in the S1 · ADP · V_i complex. In order to check this assumption we have applied the 'successive annealing' method to reveal the domain structure of S1 in the S1 · ADP · BeF_3^- complex and to compare it with the domain structure of S1 in the S1 · ADP · V_i complex. This method allows separate structural domains in a protein molecule to be revealed, i.e. the regions which melt independently from each other [6].

Table I

Thermodynamic parameters obtained from the calorimetric data for S1 and its complexes with ADP in the presence or in the absence of vanadate or beryllium fluoride

	T_d (°C)	ΔH (kJ/mol)
S1	48.4 ± 0.2	1,170 ± 80
S1 · ADP	48.9 ± 0.2	1,180 ± 80
S1 · ADP · BeF_3^-	56.5 ± 0.2	1,280 ± 90
S1 · ADP · V_i	57.8 ± 0.2	1,330 ± 90

Means ± S.E.M. are given for five different preparations studied.

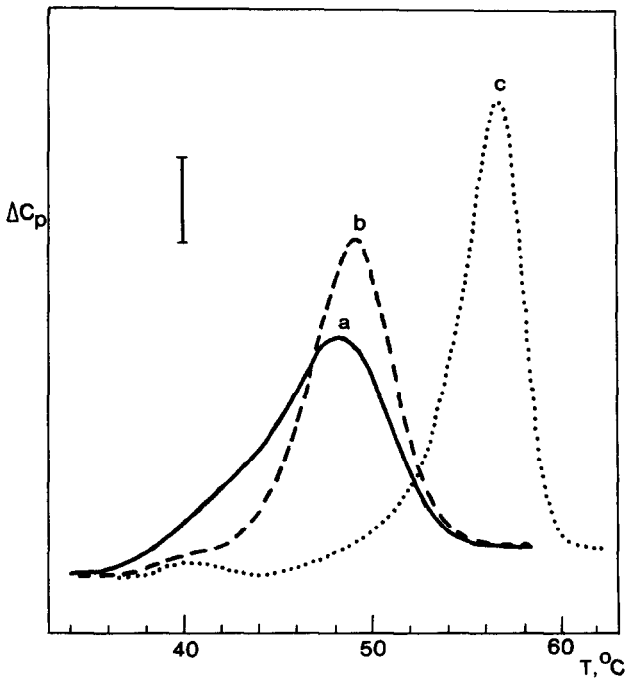

Fig. 1. Temperature dependences of excess heat capacity (ΔC_p) for (a) nucleotide-free S1, (b) S1 in the presence of 0.2 mM ADP, and (c) S1 after incubation for 30 min at 20°C in the presence of 0.2 mM ADP, 5 mM NaF and 0.2 mM $BeCl_2$. S1 concentration was 1 mg/ml. Conditions: 30 mM HEPES, pH 7.3, 1 mM $MgCl_2$. Heating rate 1°C/min. Vertical bar corresponds to 50 kJ/K · mol.

Fig. 2 shows results of a decomposition of the total heat sorption curve of the S1 · ADP · BeF_3^- complex into elementary peaks corresponding to the melting of

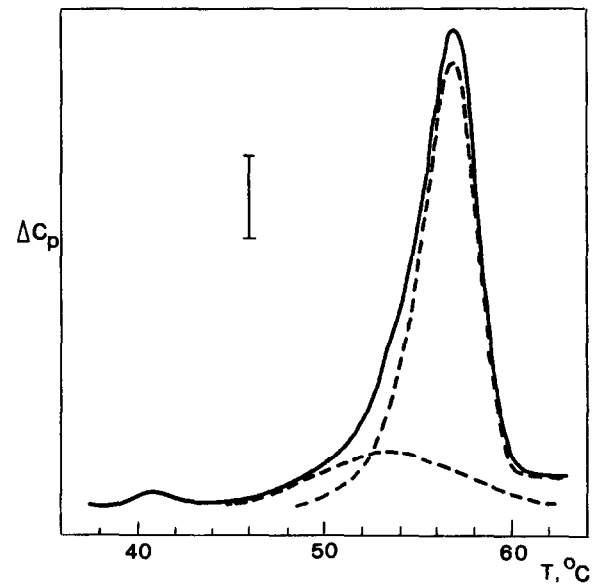

Fig. 2. Decomposition by the 'successive annealing' method of the total heat sorption curve (solid line) for the S1 · ADP · BeF_3^- complex into elementary peaks (dotted lines) corresponding to the melting of separate domains. Conditions were as in Fig. 1. Vertical bar corresponds to 50 kJ/K · mol.

separate domains. It is clearly seen that in this case S1 contains two such domains which melt with maxima at 52.7°C and 56.8°C. The calculated ΔH values were 284 kJ/mol for the first small domain and 997 kJ/mol for the second large domain. Under the same conditions S1 has a similar two-domain structure in the S1 · ADP · V_i complex (T_d = 53.0°C, ΔH = 155 kJ/mol for small domain, and T_d = 58.1°C, ΔH = 1173 kJ/mol for large domain) (data not shown). Thus, the domain structure of S1 in the complexes S1 · ADP · BeF_3^- and S1 · ADP · V_i is similar. On the other hand, it is quite different from the three-domain structure which is characteristic for nucleotide-free S1 [6,11,12] and S1 in the S1 · ADP complex [6]. This means that the formation of the S1 · ADP · BeF_3^- complex causes the conformational changes in the S1 molecule that are similar to the changes induced by the formation of the S1 · ADP · V_i complex.

There are data suggesting that the S1 · ADP · V_i complex is conformationally analogous to the intermediate M** · ADP · P_i complex of the Mg^{2+}-ATPase reaction [13,14]. Since the structure of the S1 molecule in the S1 · ADP · BeF_3^- complex is quite similar to that in the S1 · ADP · V_i complex, both these stable complexes can be considered structural analogues of the M** · ADP · P_i transition state of the myosin-catalyzed ATP hydrolysis.

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