The difference between ADP-beryllium fluoride and ADP-aluminium fluoride complexes of the spin-labeled myosin subfragment 1

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Received 21 July 1995

Abstract Electron paramagnetic resonance (EPR) spectroscopy was used for investigation of the structure of spin-labeled myosin subfragment 1 (S1) containing ADP and phosphate analogues, such as orthovanadate, aluminium fluoride (AlF_4), and beryllium fluoride (BeF_x). It has been shown that the local conformational changes in the region of Cys-707, induced by formation of the S1-ADP-BeF_x complex, differ from those of S1 containing ADP-AlF_4 or other phosphate analogues but are similar to the changes which occur in the presence of ADP or ATPγS. It is suggested that S1-ADP-AlF_4 and S1-ADP-BeF_x complexes represent structural analogues of different transition states of the ATPase cycle, namely the intermediate states S1-ADP-P_i and S1*-ATP, respectively.**

Key words: Myosin subfragment 1; Spin-labeling; Aluminium fluoride;Beryllium fluoride

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 hydrolisis. Elucidation of this energy transduction process requires the characterization of the intermediates associated with the myosin ATPase pathway. At room temperature the predominant intermediates are the M*-ATP and M**-ATP-P_i complexes (each asterisk represents an increase in intrinsic fluorescence intensity) [1–3]. Stable complexes of the isolated myosin head, or myosin subfragment 1 (S1), with ATP analogues or with ADP and phosphate analogues were used for investigation of the structure and properties of the myosin head in these intermediates.

A complex of the S1 with ADP and orthovanadate ion (V_i) was often used as stable analogue of the M**-ADP-P_i complex [4,5], and the structure of S1 in the S1-ADP-V_i complex was studied by many different techniques. It has been shown by differential scanning calorimetry and transient electrical birefringence that the trapping of ADP by V_i in the active site of S1 causes the global conformational change of the whole S1 molecule [6,7]. On the other hand, electron paramagnetic resonance (EPR) of myosin or S1 spin-labeled at SH_1-group of Cys-707 was successively used for probing of local nucleotide-induced conformational changes in this region of the head [8,9]. The conventional EPR spectrum of iodoacetamide spin-label attached to SH_1 group of S1 containing ADP-V_i was very similar to that of S1 after addition of ATP [8]; therefore it was concluded that the S1-ADP-V_i complex is a good analogue of the predominant conformational state M**-ADP-P_i.

A new phosphate analogues which form stable complexes with S1 in the presence of ADP have been recently described. These include fluoride complexes of beryllium (BeF_x) [10–12], aluminium (AlF_4) [11,12], and scandium (ScF_x) [13]. However, significant differences were found in the formation, stability and actin-induced decomposition of S1-ADP-BeF_x and S1-ADP-AlF_4 complexes [11,12,14]. These differences may indicate that the S1-ADP-BeF_x and S1-ADP-AlF_4 complexes are distinct from each other and that they mimic different structural states of the myosin head in the ATPase cycle. In the present work we have investigated the changes in the conventional EPR spectrum of spin-labeled S1 induced by the formation of the S1 complexes with ADP and various phosphate analogues. The data obtained indicate that the S1-ADP-BeF_x complex is different from the S1-ADP-AlF_4 and from all other complexes of S1 with ADP and P_i analogues.

2. Materials and methods

S1 from rabbit skeletal muscle myosin was prepared by digestion of myosin filaments with chymotrypsin [15]. Spin-labeling of S1 with 4-iodoacetamido-2,2,6,6-tetramethylpiperidinooxyl (IASL) or 4-maleimido-2,2,6,6-tetramethylpiperidinooxyl (MSL) was performed as described by Barnett and Thomas [9] Unbound spin-labels were removed by precipitation of the protein with ammonium sulfate. The extent of the modification as determined by double integration of EPR spectra [16,17] was equal to 0.7–0.8 mol of both spin-labels per mol of S1. The specificity of labeling of SH_1 group of Cys-707 was controlled by inhibition of the K^+-EDTA-ATPase and activation of the Ca^{2+}-ATPase in the presence of 0.6 M KCl. These ATPase measurements indicated that the spin-labels were almost specifically bound to the SH_1 thiol group of S1.

The S1-ADP-V_i complexes were obtained by incubation of spin-labeled S1 (10–15 mg/ml) with 2 mM $MgCl_2$, 2 mM ADP and 2 mM V_i for 30 min at 4°C in a medium containing 30 mM HEPES, pH 7.3. In order to obtain the S1-ADP-BeF_x, S1-ADP-AlF_4 and S1-ADP-ScF_x complexes, spin-labeled S1 preparations (10–15 mg/ml) were incubated for 10 min at 20°C in 30 mM HEPES, pH 7.3, 2 mM $MgCl_2$, 2 mM ADP, and 20 mM NaF; after addition of 2 mM $BeCl_2$, 2 mM $AlCl_3$, or 2 mM $ScCl_3$ the reaction mixtures were further incubated for 30 min at 4°C. Formation of the complexes was controlled by measuring Ca^{2+}-ATPase activity in the presence of 0.6 M KCl. For all complexes studies ATPase activity of spin-labeled S1 did not exceed 15% of that measured in the absence of phosphate analogues.

EPR measurements were carried out on a Varian E-104A spectrometer Spectra were recorded at 20°C with modulation amplitude of 2G and scan range of 100G, and digitized for computer treatment. Computer simulations of the EPR spectra were performed according to the model of two different motions of spin label: the fast anisotropic reorientation of the label relative to the protein molecule and the slow isotropic diffusion of the protein molecule itself [16,17] The rotational

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SSDI 0014-5793(95)00898-5

correlation time, τ, used for all simulated EPR spectra, was determined from the temperature-viscosity dependence of the separation between outer wide peaks on EPR spectra [16]. The value of τ for all spin-labeled S1 samples was 61 ± 2 ns.

3. Results and discussion

Fig. 1 represents EPR spectra of IASL-S1 in the absence of nucleotides, in the presence of ADP or adenosine 5′-*O*-(3-thiotripphosphate) (ATPγS, an ATP analogue slowly hydrolyzed by S1), and in the complexes S1-ADP-V_i, S1-ADP-BeF_x, S1-ADP-AlF_4 and S1-ADP-ScF_x. In the absence of nucleotides the EPR spectrum is characteristic of strongly immobilized spin label (Fig. 1a). The separation between the outer wide peaks, 2A′, is 68.9 ± 0.2 G for three IASL-S1 preparations studied, in agreement with previous studies on IASL-myosin [9]. Binding of ADP or ATPγS to IASL-S1 induces only a slight narrowing of the spectrum (Fig. 1b,c); the 2A′ values are 67.5 ± 0.2 G and 66.2 ± 0.2 G for IASL-S1 in the presence of ADP and ATPγS, respectively. On the other hand, formation of the S1-ADP-V_i complex results in an essential change of the spectrum which is expressed in a significant shift of spectral intensity toward the center of the spectrum (Fig. 1f). This result is in good agreement with previous EPR studies on the complexes of IASL-myosin and IASL-S1 with ADP and V_i [8,9]. Similar effects were observed for the ternary complexes of IASL-S1 with ADP and ScF_x (Fig. 1e) or AlF_4 (Fig. 1g). Formation of all these complexes induced a 10–15-G decrease in 2A′; the mean values of this parameter varied within the range 50–54 G for the S1-ADP-V_i, S1-ADP-ScF_x, and S1-ADP-AlF_4 complexes. The most pronounced effect was observed in the case of the complex S1-ADP-AlF_4 (Fig. 1g).

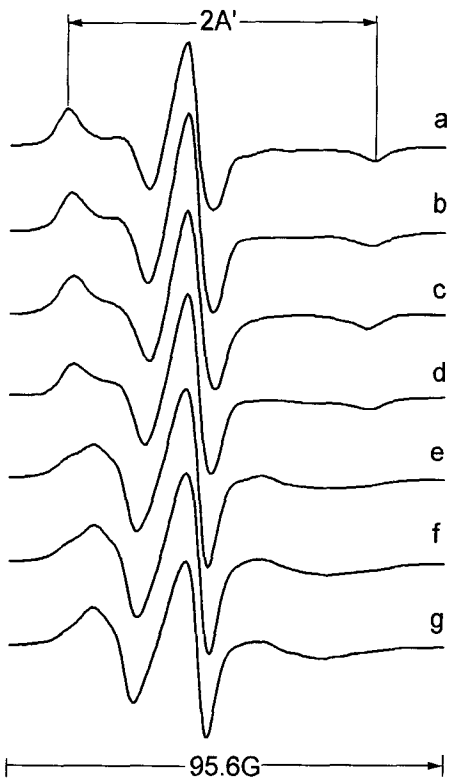

Fig. 1. EPR spectra of IASL-labeled S1 (10 mg/ml) in the presence and absence of nucleotides. (a) Without nucleotides; (b) 2 mM ADP; (c) 2 mM ATPγS; (d) 2 mM ADP and 2 mM BeF_x; (e) 2 mM ADP and 2 mM ScF_x; (f) 2 mM ADP and 2 mM V_i; (g) 2 mM ADP and 2 mM AlF_4. 2A′ is the separation between outer wide peaks.

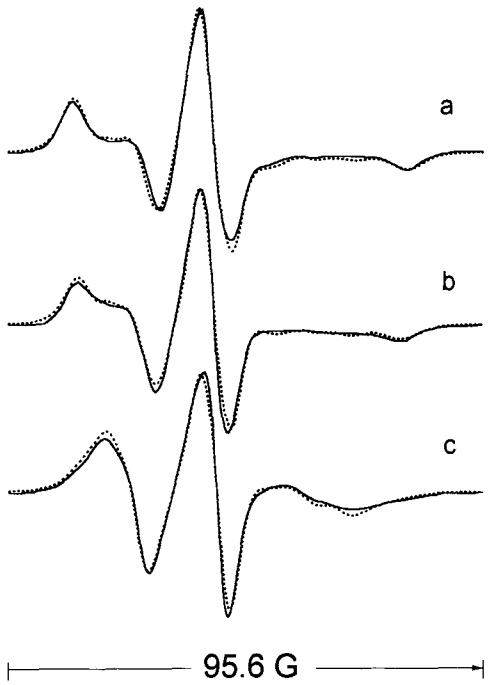

Fig. 2. The stimulated EPR spectra (dotted lines) for IASL-labeled S1 in the absence of nucleotides (a), and in the complexes S1-ADP-BeF_x (b) and S1-ADP-AlF_4 (c). Solid lines show experimental spectra.

It is surprising that the EPR spectrum of IASL-S1 in the complex with ADP and BeF_x (Fig. 1d) is similar to those of nucleotide-free S1, S1-ADP and S1-ATPγS Fig. 1a–c) and differs from the spectra of IASL-S1 in the S1-ADP-ScF_x, S1-ADP-V_i and S1-ADP-AlF_4 complexes (Fig. 1e–g). The value of 2A′ for the S1-ADP-BeF_x complex was equal to 65.0 ± 0.2 G that is close to 2A′ value of S1-ATPγS. This value is much larger than the corresponding parameter for the S1-ADP-V_i, S1-ADP-AlF_4 and S1-ADP-ScF_x complexes. This indicates that the complex of IASL-S1 with ADP and BeF_x is different from all other complexes studied. It is reasonable to assume that formation of this complex induces local conformational changes around spin label attached to SH_1 group which are different from those induced by all other complexes.

In order to characterize the IASL behavior in these S1-nucleotide complexes, we have applied the method of computer simulation and modeling of EPR spectra introduced by one of us earlier [16,17]. The parameters of theoretical spectra were varied to achieve the better coincidence of simulated and experimental spectra. The results of this fitting for IASL-S1 and its ADP-BeF_x and ADP-AlF_4 complexes are presented on Fig. 2.

Table 1
Dynamic parameters of two main states of fast motion of IASL nitroxide obtained from EPR spectra simulation.

Parameter	State 1	State 2
α	60°	78°
S (σ_s)*	0.50 (0.31)	−0.2 (0.3)
κ (σ_κ)*	−1.0 (0.36)	−1.0 (0.54)

*The values in the parentheses, σ_s and σ_κ, represent gaussian dispersions of S and κ.

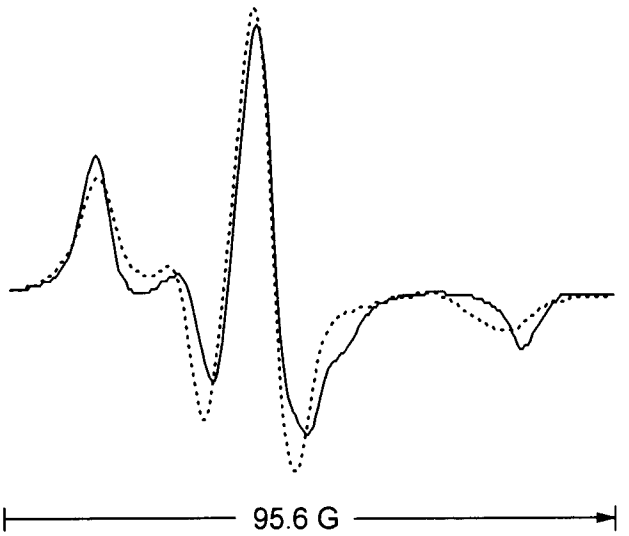

Fig. 3. EPR spectra of MSL-labeled S1 containing ADP-BeF_x (solid line) or ADP-AlF_4 (dotted line).

An analysis of simulated EPR spectra leads to conclusion that each experimental spectrum can be described as a sum of two spectra corresponding to two main states of fast motion of bound spin label. These states differ from each other by local environment of the IASL nitroxide radical. Table 1 summarizes the main parameters for these two different states: S and κ, the parameters of spatial orientation of the preferential rotation axis of nitroxide; σ_s and σ_κ, gaussian dispersions of S and κ values; α, the oscillation angle of nitroxide around rotation axes in all spin-labeled molecules. Although we detected some differences in these parameters for various S1-nucleotide complexes studied, these differences were negligible in comparison to those between two states.

Results of simulation are presented in Table 2 which shows relative content of these two states in preparations studied. In the absence of nucleotides state 1 predominates, whereas in the S1-ADP-AlF_4 complex the contribution of state 1 is negligible and state 2 is a predominant one. On the contrary, S1-ADP-BeF_x, like S1-ADP and S1-ATPγS, is characterized predominantly by state 1. These data agree with postulate that the local environment around SH_1-group of Cys-707 in S1-ADP-AlF_4 and S1-ADP-BeF_x complexes is different.

Similar experiments were carried out with S1 specifically labeled at SH_1 group by maleimide-based spin-label (MSL). MSL bound to SH_1 is much less sensitive than IASL to local conformational changes induced by ADP and V_i addition [8,9]. However, in this case we also observed a rather pronounced difference between EPR spectra of the S1-ADP-BeF_x and S1-ADP-AlF_4 complexes (Fig. 3). The value of 2A′ for S1-ADP-AlF_4 was reduced by about 2 G compared with S1-ADP-BeF_x, whereas the difference in this parameter between S1-ADP-BeF_x and S1-ADP was less than 0.5 G (data not shown). Thus, both IASL and MSL show the difference between S1-ADP-BeF_x and S1-ADP-AlF_4 complexes

The data obtained lead to conclusion that formation of the S1-ADP-AlF_4 complex results in a significant conformational change of the region around Cys-707, while formation of the

Table 2
Contribution of two motional states into EPR spectra of IASL-labeled S1 and its complexes with nucleotides

Sample	State 1	State 2
S1	0.84	0.16
S1 + ADP	0.74	0.26
S1 + ATPγS	0.70	0.30
S1 + ADP + BeF_x	0.72	0.28
S1 + ADP + ScF_x	0.26	0.74
S1 + ADP + V_i	0.22	0.78
S1 + ADP + AlF_4	0.10	0.90

S1-ADP-BeF_x complex causes only slight changes in this region of the S1 molecule. Recently published data on the three-dimensional structure of a truncated myosin head from *Dictyostelium discoideum* myosin II also indicate significant conformational changes in the region corresponding to that of SH_1 of skeletal muscle S1 induced by formation of the complex with ADP-AlF_4 and the absence of such changes in the complex with ADP-BeF_x [18]. Moreover, we have shown that the effect of ADP-BeF_x is similar to that of ATPγS (Fig. 1, Table 2). Therefore our data support the idea that S1-ADP-AlF_4 and S1-ADP-BeF_x complexes may represent analogues of two different states in the ATPase cycle, with the former resembling the S1**-ADP-P_i state and the later resembling the S1-ATP [19].

Acknowledgements. This work was supported in part by NIH Grant 1 R03 TW00270-01, Grants SBH000, MEI000 and MEI300 from the International Science Foundation, and Grant 94-04-12148 from Russian Fund of Fundamental Research.

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