A CIRCULAR DICHROISM STUDY OF CARDIAC MYOSIN AND ITS INTERACTION WITH ADP

Susan J. SMITH
Department of Physiology, University College London, Gower Street, London, WC1E 6BT, England

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1. Introduction

Rabbit skeletal myosin has circular dichroism which displays in the near ultraviolet a system of Cotton effects associated with some aromatic residues [1–4]. This aromatic circular dichroism is perturbed when ligands bind at the active site presumably reflecting an interaction of the ligand with tyrosine and/or tryptophan side chains. Perturbations in the ultraviolet absorption spectrum [5,6] and in the fluorescence [7,8] may be seen as other manifestations of the same interaction. This phenomenon has not, however, so far been found to occur in other myosins, and in particular the examination of a range of invertebrate myosins [9] has shown that the spectroscopically active aromatic residues present in or near the active centre of rabbit skeletal myosin is not present in the other species.

In rabbit skeletal myosin and its active fragments the change in aromatic circular dichroism affords a method of following the binding of active centre ligands, and the analysis of binding profiles [4]. Such a study has given results consistent with the presence of two functional and spectroscopically indistinguishable sites per myosin molecule [4], rather than two sites of grossly different spectroscopic and thermodynamic characteristics as has been claimed [5,6]. In this report the behaviour of bovine cardiac myosin is described. The results show first that the protein has a system of Cotton effects arising from aromatic residues, similar to but smaller than those of skeletal myosin; that they are subject to a similar, though again smaller, perturbation by ligands; and that the binding of ADP, as followed by the change in circular dichroism, reflects the presence of two functional sites, which are equivalent within the limits of precision of method.

2. Materials and methods

Bovine cardiac myosin was prepared essentially by the method in [10] and was purified by ion exchange chromatography [11]. Cardiac subfragment 1 was prepared and purified as in [12]. Circular dichroism was measured on Jouan CD III and Jasco JV40 instruments. Absorbance levels were kept below 1.2. Scanning speeds of 1 nm/min at a pen period of 16 s were used; where necessary a small correction was made for the contribution of ADP itself. Titrations were performed at fixed wavelength (280 nm). The temperature was maintained within 1°C ± 12°C by circulation of thermostat fluid through the cell block.

3. Results

Figure 1 shows the circular dichroism in the region of aromatic absorption of bovine cardiac myosin and its subfragment-1. Progressions of Cotton effects, attributable to both tryptophan [13] and tyrosine [14] residues can be identified. The pattern resembles that given by rabbit skeletal myosin, but is of half the amplitude. The effects of introducing ADP and pyrophosphate are also shown in fig.1. Assuming that the circular dichroism of bound ADP is the same as that of free, it appears that all but the two longest-wave-

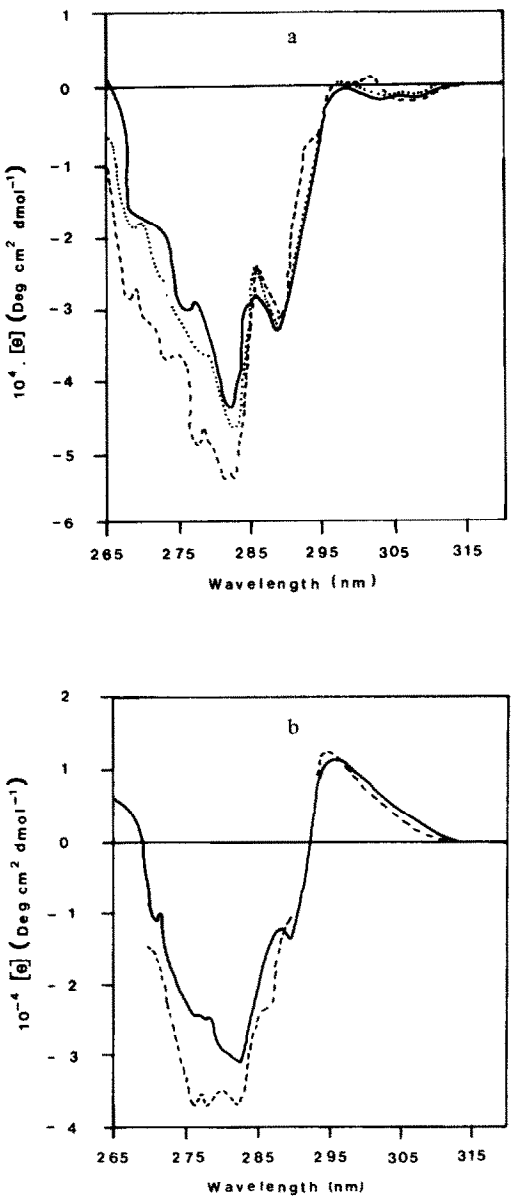

Fig.1.(a) Circular dichroism (referred to mol. protein) of cardiac myosin (———) with excess ADP (– – – – –), with excess pyrophosphate (. . . . . .) at 12°C. Solvent: 0.5 M KCl, 10 mM Tris–HCl, 5 mM $MgCl_2$ (pH 8.0), 1 μM myokinase inhibitor [p^1, p^5-di(adenosine-5^1-)pentaphosphate]. The contribution of ADP has been subtracted. (b) Circular dichroism (referred to mol. protein) of cardiac subfragment 1 (———), with excess ADP (– – – – –) at 12°C. Solvent: 0.1 M KCl, 10 mM Tris–HCl, 5 mM $MgCl_2$ (pH 8.0), 1 μM myokinase inhibitor [p^1, p^5-di(adenosine-5^1-)pentaphosphate]. The contribution of ADP has been subtracted.

length extrema are affected by this ligand. Pyrophosphate leaves the three extrema of longest wavelength undisturbed. If the contributions of the adenosine and diphosphate moieties of the ADP are additive in their perturbing effect on aromatic residues at the subsites, these two contributions can be separated; that of the adenosine being confined to the region between 270 and 290 nm. Again this is very similar, at least in qualitative terms, to the behaviour observed with rabbit skeletal myosin [4].

Figure 2 shows the binding profile, as observed by circular dichroism of ADP to cardiac myosin, and fig.3 that to cardiac subfragment-1. The binding curves in either case indicate a titre of active myosin heads in the range 85–90%. These curves are therefore most simply interpreted in terms of one type of site, functioning independently. The binding constants in the solvent and temperature conditions of these experiments are in the range 10^6–10^7 M^{-1} as shown, which agrees with the results of studies by other methods, viz. microcalorimetry [13] and proton release [14]. The method provides a means of obtaining a titre of active sites, and the results here show that the subfragment-1 prepared from cardiac myosin is substantially active.

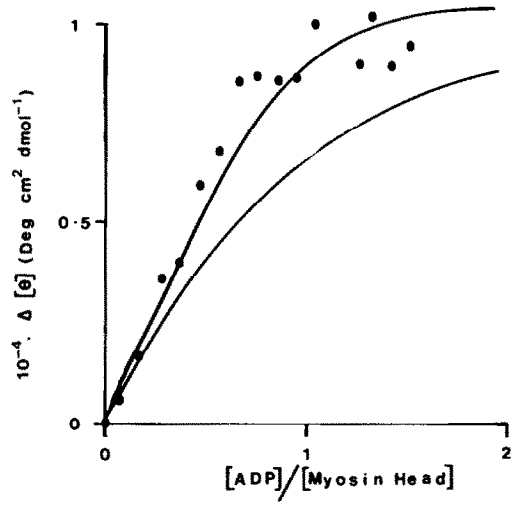

Fig.2. Typical circular dichroism titration of cardiac myosin with ADP; the ordinate is the circular dichroism difference generated at 280 nm. The curves are calculated for identical sites with association constants (reading downwards) of 10^7 and 10^6 M^{-1}. Experimental conditions as described in legend to fig.1(a).

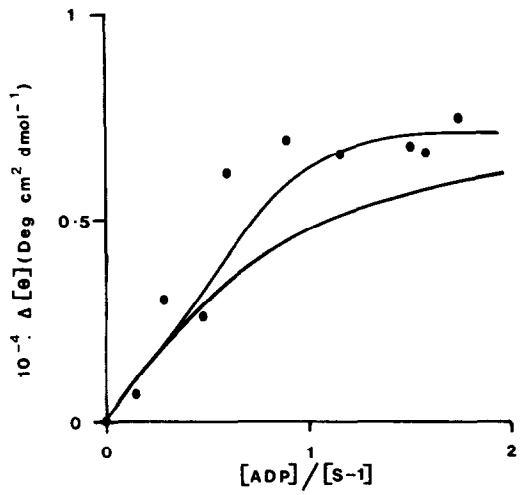

Fig.3. Typical circular dichroism titration of cardiac subfragment 1 with ADP. The curves are calculated for identical sites with association constants (reading downwards) of 10^7 and 10^6 M^{-1}. Experimental conditions as described in legend to fig.1(b).

4. Discussion

The circular dichroism of cardiac myosin and its perturbation by ADP and pyrophosphate indicates that the active centre has close structural similarities to that of rabbit skeletal myosin, both proteins differing by this criterion from invertebrate myosins, which show no such optical perturbations on binding of ligands [9]. The small amplitude of the aromatic circular dichroism compared with that of skeletal myosin probably reflects only small differences in side chain orientation, to which the induced optical activity is very sensitive [17]. The similarity of the binding profiles for ADP to those observed for skeletal myosin is consistent with the broadly similar ATPase mechanism in the two enzymes [12].

The presence of two sites on each myosin molecule, giving identical spectroscopic response, and being identical, or at least very similar, in ligand affinity, is in agreement with two studies by other methods [15,16]. There is no indication of the spectroscopic (structural) heterogeneity reported to exist in skeletal myosin [5,6], and even though the circular dichroism experiments on the intact myosin were carried out at high ionic strength, there is no evidence for the presence of two species of binding site, differing substantially in affinity in these conditions only, as again reported for skeletal myosin [14,17]. This is presumably because the type of heterogeneity known to be present in rabbit skeletal muscle myosin [19–21] is absent in the cardiac type, as reflected for example in the presence of only one species of alkali light chain [19]. The data here reported are not, however, of sufficient precision to exclude the possibility of appreciable cooperativity by virtue of head–head interaction, for as has been shown [4], the demonstration of such an effect in a system, in which the protein and ligand concentrations are necessarily not very different in magnitude, is very difficult. A measure of cooperativity cannot therefore at this stage be excluded.

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